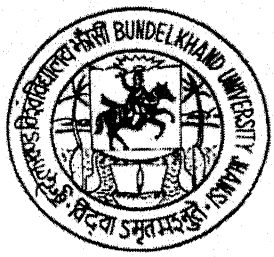
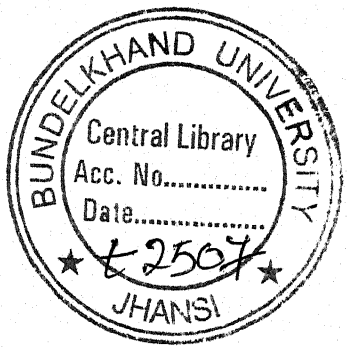
CONTROLLED RELEASE SYSTEMS OF ORAL CEPHALOSPORIN ANTIBIOTIC

Thesis Submitted To The

BUNDELKHAND UNIVERSITY
JHANSI





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IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

(PHARMACEUTICAL SCIENCES)

SEPTEMBER 2003

CERTIFICATE

This is to certify that the thesis entitled "Controlled Release System of Oral Cephalosporin Antibiotic" submitted in fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY (PHARMACEUTICAL SCIENCES) is a record of bonafide research carried out by Himadri Sen at Lupin Research Center, Aurangabad and Lupin Research Park, Pune, under our supervision and the manuscript is suitable for submission for the award of degree of DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL SCIENCES.

This is to further certify that Himadri Sen has put in minimum 200 days attendance during the course of this study.

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ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude to my guide Prof. Ramesh Chandra, Vice Chancellor, Bundelkhand University, Jhansi for his invaluable support and generous assistance in fulfillment of the Ph.D. program.

I thank Dr Sudershan K Arora, President R&D, Lupin Research Park, Pune, for his persistence and motivation to complete this program.

I am grateful to Dr D. B. Gupta, Chairman and the management of Lupin Limited, for fulfilling my desire in converting this industrial project to an intellectual capital by allowing the use of laboratory facilities. I am also very thankful to the scientists of Pharma Research for their invaluable support.

I remain indebted to Dr Gita Rao, for her support and co-operation towards the compilation of this Ph.D. thesis.

Finally, I acknowledge my wife, Ranjini Sen, my parents (Mrs Prativa Sen and Late Dr D.B. Sen) and my son, Niladri Sen for their constant encouragement and moral support.

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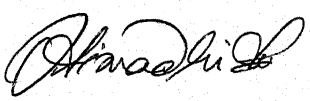

(HIMADRI SEN)

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INTRODUCTION

The Indian pharmaceutical industry has grown significantly after independence. The most important event that helped this industry was the enactment of Indian Patents Act of 1970. With only the process patent being allowed, various drugs, intermediates and chemicals were prepared by shorter synthetic routes than originally reported by the inventor, within a few months, by highly cost effective process. As a result, Indian industries made new drugs available at much lower costs than anywhere in the world. This brought a revolution in the Indian drug and pharma industrial sector.

In 1995, with the GATT agreement, Government of India has agreed to implement product patent with effect from January 2005 and this has got the Indian pharmaceutical industry at the crossroads. It is imperative that none can make drugs originally discovered and patented by others even by innovative new process without paying royalties to the inventor. The increasing national and international competition, the collapse of geographical barriers, changing world markets, liberalization and globalization would radically influence the direction of the industry.

Post 2005, survival will largely be governed by fundamental strengths in developing new chemical entities (NCEs). The average cost of developing an NCE is 350million USD with a time span of 10-12 years and a success rate of 1 in 10,000[1]. Considering the cost and the uncertainty of new drug discovery, an alternative strategy would possibly be to produce and competitively exploit technologically advanced products in new areas with demonstrable benefits. This strategy would include the development of novel drug delivery systems (NDDS), an area hitherto neglected by the industry due to lack of product patents.

1.1 Novel Drug Delivery Systems[2]

Novel drug delivery systems are technologies that aid or enable the better administration of therapeutic compounds. These systems include devices, such as inhalers or transdermal patches, as well as formulation technologies. The average cost of developing a NDDS is 2-8 million USD with a time span of 2-8 years and a medium to high success rate. In the US, a separate industry has arisen that focuses on the improvement of drug delivery systems of existing drug molecules. The market impact of formulating a oral controlled release system (OCRS) for an existing conventional product is shown in Table 1.1

Table 1.1 Market impact of conventional V/s OCRS switching

Product	**6 months**	**1 year**	**2 years**
Effexor	68%	43%	23%
Effexor XR	32%	57%	77%
Lodine	59%	42%	17%
Lodine XL	41%	58%	83%
Tegretol	86%	76%	63%
Tegretol XR	14%	24%	37%
Voltaren	45%	34%	20%
Voltaren XR	55%	66%	80%
Average- Twice a day	65%	49%	31%
Averge-Once a day	35%	51%	69%

Since R.P. Scherer and KV Pharmaceuticals were founded in the pre-World War II era, more than 100 companies have been actively involved in developing novel drug delivery systems, and the industry is growing at a considerable pace. The worldwide market growth of novel drug delivery systems is shown in Table 1.2.

Table 1.2: Worldwide market growth in novel drug delivery segments (in $ in billions)

Technology	2000	2005	Growth
Controlled release	14.2	26.3	85%
Pulmonary, inhalation	11.7	22.6	93%
Transnasal delivery	8.2	16.0	95%
Transmucosal	2.4	6.5	171%
Transdermal delivery	6.7	12.7	90%
Injectable/implantable	3.8	7.2	89%
Needle-less injection	.4	1	150%
Rectal	.5	1.2	140%
Liposomal	1.2	3.3	175%
Cell/gene therapy	0	5	0%
Miscellaneous	1.5	2.5	67%
Total	50.6	104.3	106%

According to the investment bank Dillon Read & Company, the novel drug delivery system market will grow from $ 11.5 billion, or 12% of the total pharmaceutical market in 1996, to $35 billion, or 20% of the total pharmaceutical market in 2005. Drug companies need to use a range of technologies to reformulate their products, as each drug represents different technical challenges and different unmet clinical needs. With the availability of new polymers and better understanding of how to circumvent first pass metabolism, drug delivery companies in developed countries, apply their technologies across a range of clinical segments. The challenges in drug delivery will be multiplied for delivering biotechnology based products, which due to their poor solubility, bioavailability, stability and extensive first pass have conventionally been delivered by the invasive injectable route, by non-invasive routes.

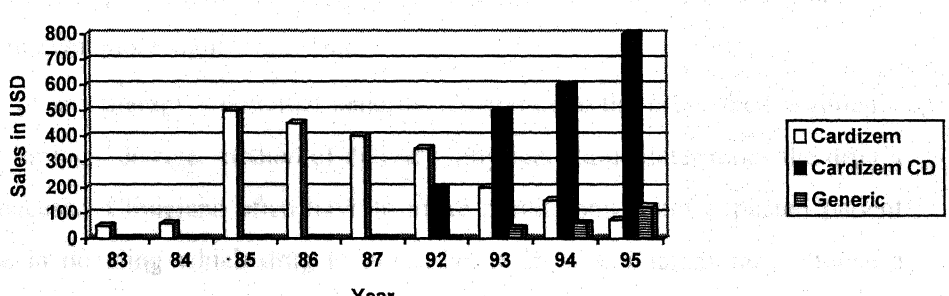
1.1.1 Advantages of Novel Drug Delivery Systems[2]

The sources of value in reformulation include extending patent life, patient convenience /compliance improvement, improved therapeutic efficacy, reduced manufacturing costs, and market share expansion.

1.1.1.1 Value of patent extension

Because patents guarantee market exclusivity and artificially high premiums, patent expiration translates into rapidly declining sales for brand-name pharmaceuticals. In US, generic versions of drugs, are typically introduced at 20-25% of the branded drugs' prices. The branded drug's market erodes rapidly and loses 60-80% of the total days of therapy within 6 months- the influence of managed care and mandatory substitution laws. For example, after Tagamet went off-patent in May of 1994, SmithKline Beecham retained only 34% of the sale of cimetidine in 1995. Reformulating a branded drug may create an improved version that is preferred by clinicians and patients over the less expensive generic versions of the original branded product. Patents on the reformulated product allow the company to effectively extend the patent life of the drug. This strategy can dramatically affect the branded drug's market share. A typical example is that for Diltiazem. This drug was discovered by Hoechst Marion Roussel and patent on its product, Cardizem, expired in 1988. It was reformulated as Cardizem CD by a drug delivery company, Mylan, a once-daily version of the drug, in 1992. Inspite of the launch of a generic version of the conventional drug in 1993, the company retained 86% of sale of diltiazem after patent expiration sheerly due to the CD version as can be seen from Fig. 1.1.

Fig 1.1: Life cycle extension of Diltiazem by developing a controlled delivery system



1.1.1.2 Value of Convenience (frequency of dosing) & Compliance Improvement

In terms of the frequency of administration, less is more. Drugs that must be taken only once per day are ideal, because they gain the highest compliance. Compliance has been shown to drop off sharply for drugs that have to be taken more than three times per day, thus drugs with more frequent dosing schedules are generally considered unacceptable for therapies that must be taken chronically.

A sustained release oral formulation that allows for once per day dosing is the preferred form of drug delivery. Aerosol formulations have not been as convenient as oral because they have often required frequent (3 or more times per day) dosing. In addition, the effectiveness of aerosol formulations has been hampered by the inconsistency of inhalers, which results in inadequate or varying levels of drug absorption. Nasal delivery has also lacked consistency in dosage absorbed due to backflow of drug after administration and variations in nasal architecture and volume of mucus between patients. Transdermal systems although usually providing dosing from three to seven days, are perceived by patients as less attractive because patches can result in skin irritation and may not adhere to the skin efficiently. Depot injections offer significant improvement over frequent injections or intravenous infusions.

One of the most significant impediments to keeping patients healthy and curing disease is noncompliance with prescribed medication regimens. Noncompliance explains why therapies often demonstrate greater efficacy in closely monitored clinical trials than in routine medical practice. Although compliance with prescribed regimens is not a significant issue in the closely supervised hospital setting, it is one of the most important issues for outpatients. With the average length of stay in hospitals decreasing and patients being released in a less stable state, compliance is becoming an even more significant issue.

Regardless of any therapy's potential benefits, adherence to the prescribed regimen – the correct timings, dosage, method of delivery, physical status-determines the drug's ultimate success. Clinicians often have to make predictions about expected patient compliance in deciding which drug to prescribe, if any. Clinicians may choose a suboptimal drug for fear that compliance on the more appropriate therapy will be low.

Many factors influence patient compliance, including the nature of the disease and disease symptoms, cognitive or functional ability, and financial resources. Some important factors influencing compliance, the frequency and mode of administration and the extent of drug-related side effects, can be modified through drug reformulation.

1.1.1.3 Mode of Administration

Clinicians have learned that to achieve high patient compliance, in the absence of serious noncompliance penalties, drug regimens must be convenient and uncomplicated. Inconvenient (injectables) or complex (many dosage per day) regimens lead to poor compliance.

The oral formulation is the most preferred mode of administration as it is the easiest form for patients to tolerate. Fig 1.2 shows that 76% of the market value for top selling 100 conventional drug products in US comes from the oral systems. Similarly, as shown in Fig. 1.3, among the drug delivery systems, oral drug delivery systems contribute a major portion of the pie.

Fig 1.2: Top 100 Drugs- U.S. Market Value

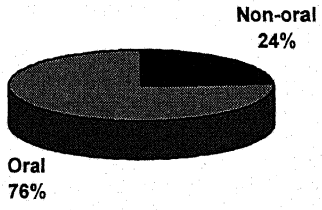
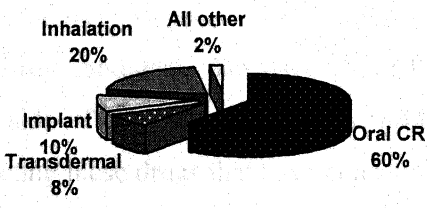


Fig 1.3: Types of Drug Delivery Systems



The most successful drug delivery formulations have been, as would be expected, oral sustained release formulations. Currently, Bayer AG's Adalat CC product for hypertension leads the oral sustained –release market. Reformulated in 1993 from a 3-4/day to 1/day, Adalat CC has climbed the sales of $ 1.1 billion worldwide in 1997. Another highly successful reformulation has been TAP Pharmaceuticals' Lupron Depot for prostate cancer and endometriosis. Reformulated from a daily injection in 1989 to a once per month injection, Lupron Depot has climbed to worldwide sales of $ 990 million in 1997.

1.1.1.3 Value of Reduced Manufacturing Costs

One method of increasing profitability on a drug is decreasing manufacturing costs. Often orally formulated drugs with poor bioavailability must be administered in high doses, because only a small percentage of the active ingredients is absorbed by the body. Drug reformulations that improve the bioavailability of the drug require less active ingredient to produce an equivalent therapeutic effect, thereby reducing manufacturing costs.

Maximizing the bioavailability of these types of agents will be rewarded in the market.

1.2 Selection of drug candidates[2]

One of the most critical components of a drug or drug delivery company's strategy is the choice of which compounds to reformulate. This process should include the following explicit steps: developing a starting list of drug candidates, examining the delivery technology, assessing the therapeutic or administrative unmet needs, performing a competitive screen, and sizing the market .

Because there is considerable value in extending the patent life of a compound, drug companies often focus on their blockbuster drugs that are coming off-patent for developing novel drug delivery systems. The pharmaceutical industry's "graveyard" is another source of drug candidates that have clinical potential but have failed in clinical trials due to side effects or administrative problems. There may be even more value in resurrecting these drugs that have consumed costly drug approval and / or market acceptance without reformulation.

The sophistication of novel drug delivery technologies is advancing rapidly and companies' success rates are growing. As a result, in the longer term, pharmaceutical companies, will proactively elect to reformulate drugs well before they reach their patent maturities. As a recent example, Pfizer announced that is was teaming with R.P.Scherer to reformulate a faster-acting form of its blockbuster Viagra less than 4 weeks after it launched the drug. In fact, as drug delivery matures as a science, pharmaceutical companies will involve the technologies in their initial formulations.

1.2.1 Therapeutic rationale for formulating OCRS of β-lactum antibiotics

Orally absorbed Cephalosporins have been in clinical use for almost thirty years. Cephalosporins, belonging to the group of β-lactam antibiotics, are bactericidal and they act by inhibiting the bacterial cell wall. Cephalosporins are classified by generation, which is primarily based on the general features of their antibacterial activity. Succeeding generations generally have increasing activity against Gram-negative bacteria. Although the number of available oral agents is increasing, it is still small in comparison with the injectable Cephalosporins. Examples of currently marketed orally active Cephalosporins are Cefuroxime axetil, Cefixime trihydrate, Cefadroxil, Cefpodoxime Proxetil , Cefatrizine, Ceftibuten, Cephalexin, Cephradine and Cefaclor. It is interesting to note that these compounds belong to different generations. It will therefore rapidly be recognized that due to the relative scarcity of these compounds it is important to further explore the possibilities to improve dosage-forms containing such antibiotics. e.g. Cefaclor, a second generation product, has been marketed in the United States of America since 1979. It still widely used broad spectrum antibiotic having a good activity after oral administration both against Gram-positive cocci and Gram-negative bacteria. It is primarily used in the treatment of upper and lower respiratory tract infections, but urinary tract, skin and soft tissue infections have also been successfully treated with the compound. In view of this activity against Haemophilus influenzae it is very suitable for the treatment of infections such as otitis media. Due to the short serum elimination half life of ≤ 1 hour, the usual adult dose for Cefaclor is 250 to 500 mg every 8 hours. For current therapeutic practice, Cefaclor is commercially available as a capsule, modified release tablet, suspension and as a dispersible tablet. The commercial availability of such different dosage-forms reflects the prescriber's need to select the most appropriate dosage-form for each individual patient.

The selection of the appropriate mode of drug administration is of major importance since the efficacy of the drug greatly depends thereon. The selection should be based upon the pharmacokinetic and pharmacodynamic properties of the drug. While emphasis should be given to pharmacokinetic parameters, such a absorption characteristics, protein binding and clearance, less concern is given to the pharmacodynamic profile of the drug i.e. the concentration-effect relationship.

The relationship between drug concentration and its inhibitory effect on microbial growth for a certain drug-pathogen combination can be determined *in vitro*. While in certain cases (e.g. aminoglycosides) elevation of drug concentration is associated with enhanced bactericidal potency, other cases (such as β-lactam antibiotics and erythromycin) are not highly concentration-dependent. β-lactam antibiotics exhibit minimal concentration dependent killing and produce short term effect with most of the bacteria[3] . The killing rate of these antibiotics saturates at concentration of around 4-5 times the minimal bactericidal concentration. Thus, high concentration will not kill bacteria faster than lower concentration[4]. The post-antibiotic effect (PAE) of the drug is another pharmacodynamic parameter that has to be taken account for the determination of an optimal dosage regime. β-lactam antibiotics exhibit minimal PAE.

Hence the goal of the dosage regimen of β-lactam antibiotics should be to prevent the drug free interval between doses from being long enough for the bacterial pathogen to resume regrowth[5]. This can be achieved by providing long acting pharmaceutical composition, which would maintain low but effective concentrations for a prolonged period thus improving patient compliance / convenience along with reduction in fluctuations in drug level thus improving clinical efficacy of the antibiotic[6] .

Depending on the $t_{1/2}$ of the antibiotic, time over MIC(T>MIC) can be extended in two ways:

a) by increasing its dose eg cefixime (from 200mg for BID to 400mg for OD), Cefdinir, ceftibuten and amoxicillin(500mg TID to 1.75mg BID). The disadvantage of this approach is that the Cmax is also increased.

b) by formulating an oral controlled release product having Cmax matching to the immediate release formulation and AUC equivalent to immediate release formulation administered BID/TID e.g. cephalexin, cefuroxime axetil and cefaclor.

1.3 Objective of present study

Antibiotics, including cephalosporins, have traditionally been formulated as dose dependent delivery systems. The multiple frequency of dosing, leads to peak and trough profile, poor patient compliance, convenience and results in drug resistance in many cases. To overcome drug resistance, expensive and time consuming, discovery of newer generations of anti-infectives has been imperative.

In the recent past, drug delivery interventions are moving more and more from "dose dependent" to "rate-controlled" release systems for both, newer and older drugs depending on factors like solubility, permeability, biological half-life etc. There are many examples of this approach such as:

a) Nifedipine OD formulations introduced in the last decade
b) Ceclor CD has changed the frequency of dosing from TID to BID
c) Amoxycillin 875mg to switch from TID to BID
d) Ciprofloxacin OD to switch from BID to OD

Such interventions have resulted in better patient convenience and compliance, better clinical outcome, reduction in side effects and minimized drug resistance. This approach is less risky and requires a shorter period of time than inventing a new chemical entity.

The objective of the present study is to design, a patent non-infringing, stable, OCRS-BID, bioequivalent with Ceclor CD, 500mg and a novel, stable and bioavailable OCRS-OD for β–lactum antibiotic, Cefaclor.

2

LITERATURE SURVEY

For many decades now, the conventional drug delivery systems i.e. tablets, capsules, pills, suppositories, creams, ointments, liquids aerosols, injectables are the primary pharmaceutical products commonly seen in the prescription and OTC products which provide prompt release of the drug to achieve and maintain the drug concentration within therapeutic range needed for treatment. Several potential problems are associated with this approach:

a) Unless the dosing interval is relatively short, depending on the biological half life of the drug, large peaks and valleys in the drug level occur. Oscillations in drug levels may be understandable in some diseased conditions.

b) Success by this approach is dependent on patient compliance with the dosing regimen. Numerous studies have documented that lack of compliance is an important reason for drug therapy inefficiency or failure.

c) During the early periods of dosing there may be insufficient drug to generate a favorable biological response, which may be a significant problem in certain diseased states.

d) For drugs with short biological half-lives, frequent dosing is needed to maintain relatively constant therapeutic levels of the drug.

It is often necessary to take this type of drug delivery system several times a day resulting in significant fluctuation in the drug plasma level. Recently, several technical advancements have been made which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity, and /or targeting the delivery of drug to a tissue. These advancements have led to the development of several novel drug delivery systems that have revolutionalized the method of medication and provide the following therapeutic benefits[7]:

a) Better patient compliance/convenience

Table 2.1: Frequency of dosing Vs % compliance

FREQUENCY OF DOSING	PERCENT COMPLIANCE (All medication taken as directed)
o.d	90 %
b.i.d.	69 %
t.i.d.	38 %

b) Employ less total drug

- Minimize or eliminate local side effects
- Minimize or eliminate systemic side effects
- Obtain less potentiation or reduction in drug activity with chronic use
- Minimize drug accumulation with chronic dosing

c) Improve efficiency in treatment

- Cure or control condition more promptly
- Improve control of condition i.e. reduce fluctuation in drug level
- Make use of special effect e.g. sustained release aspirin provides sufficient drug, so that on awakening the arthritic patient has symptomatic relief

2.1 Oral controlled release drug delivery systems (OCRS)[8,9]

Oral route is the most widely utilized route of administration for systemic delivery of drugs via various pharmaceutical dosage forms. In general sense, the gastrointestinal tract is a hostile environment which must be contended within product design. The oral route is a relatively safe route for sustained release drug delivery and offers less potential dangers than any other routes. Thus although the constraints of the oral route are numerous, and at times severe, there is still more flexibility in dosage form design, by this route, than exists for other routes.

All the pharmaceutical products formulated for systemic delivery, via the oral route of administration, irrespective of mode of delivery, (immediate, sustained, or controlled release) and the design of dosage form (either solid , dispersion or liquid) must be developed within the intrinsic characteristic of G.I. physiology. Therefore the fundamental understanding of various disciplines including G.I. physiology, pharmacokinetics, pharmacodynamics and formulation design is essential to achieve a systematic approach to the successful development of an oral pharmaceutical dosage form. Although it is often impractical to alter the physicochemical, pharmacokinetic and pharmacodynamic characteristics of a drug to be delivered by a chemical approach, such as synthesis of analog, or medically undesirable to modify the anatomic and physiological characteristic of the G.I.T., the design of controlled release of oral dosage form by optimization of dosage form characteristic with G.I. anatomy and physiology taken into consideration could provide some opportunity to rationalize systemic delivery of drugs and maximize their therapeutic benefits. Thus the controlled release drug administration means not only prolongation of drug delivery time but continuous delivery of drug at predictable and reproducible kinetics for a predetermined period through out G.I. transit.

A review of the literature has revealed the following Novel Drug Delivery systems that can be utilized for the controlled delivery of drugs in the alimentary canal :

a) Dissolution controlled release systems
   i) Matrix (or monolithic) dissolution controlled release
   ii) Encapsulation / coating dissolution controlled system (Reservoir devices)

b) Diffusion controlled release systems
   - Matrix diffusion controlled system
     i) Non-swellable hydrophobic matrix (rigid matrix)
     ii) Swellable hydrophilic substance
   - Reservoir devices (or Laminated devices)

c) Dissolution and diffusion controlled release systems

d) Osmotic pressure – controlled gastrointestinal delivery systems e.g. Acutrim tablets achieve 16 hrs oral controlled delivery of Phenyl propanolamine

e) Hydrodynamic pressure – controlled gastric intestinal delivery systems

f) Membrane permeation controlled gastro intestinal delivery systems
   - Micro porous membrane permeation controlled gastrointestinal delivery systems
   - Gastric fluid resistant intestine targeted controlled release delivery systems

g) Gel diffusion intestine targeted controlled release delivery systems

h) pH controlled intestine targeted controlled release delivery systems

i) Ion exchange intestine targeted controlled release delivery systems e.g. Pennkinetic system (Pennwest Pharmaceuticals) (sustained release suspension)

j) Prolongation of G.I. retention
   i) Hydrodynamically balanced intragastric delivery systems
   ii) Intragastric floating gastrointestinal drug delivery systems
   iii) Inflatable gastrointestinal controlled drug delivery systems
   iv) Ingestric osmotically controlled drug delivery systems
   v) Intrarumen controlled drug delivery systems
   vi) Bio(muco)adhesive controlled delivery systems
   vii) Co-administration with GI motility reducing drugs

k) Overcoming hepatic first pass metabolism
   i) Oral mucosal drug delivery
   ii) Rectal mucosal drug delivery

Because of their relative ease of production and cost, compared with other methods of sustained or controlled delivery, dissolution and diffusion-controlled systems have classically been of primary importance in oral delivery of medication.

2.1.1 Matrix diffusion controlled systems [10]

A matrix device consists of drug dispersed homogeneously throughout a polymer matrix. The drug in the outside layer exposed to the dissolution medium is dissolved first and then diffuses out of the matrix. The process continues with the interface between the dissolution medium and the solid drug moving toward the interior. For this system to be diffusion-controlled, the rate of dissolution of drug particles within the matrix must be much faster than the diffusion rate of dissolved drug leaving the matrix.

Matrix systems offer several advantages. They are, in general, easy to make. Since the drug is dispersed in the matrix system, accidental leakage of the total drug component is less likely to occur, although occasionally, cracking of the matrix material can cause unwanted release. The primary disadvantages of this system are that the remaining

matrix ghost must be removed after the drug is released. Also, the release rates generated are not zero-order, since the rate varies with the square root of time. A substantial sustained effect however can be produced through the use of very slow release rates, which in many applications are indistinguishable from zero-order. A list of matrix diffusional products is shown in table 2.2 below:

Table 2.2: Matrix diffusional products

Product	Drug substance	Manufacturer
Desoxyn-Gradumet	Methamphetamine hydrochloride	Abbot
Fer-Gradumet	Ferrous sulphate	Abbot
Tral Filmtab	Hexocyclium methylsulfate	Abbot
PBZ-SR	Tripelennamine	Geigy
Procan SR	Procainamide hydrochloride	Parke-Davis

Matrix diffusion controlled systems are classified into two types[11]:

a) Non swellable matrix where the drug is dispersed in an insoluble matrix of rigid nonswellable hydrophobic material such as PVC, fatty materials like Stearic acid and Bees wax.

b) Swellable hydrophilic matrix: These systems are popular for sustaining highly water soluble drugs. The materials used for such matrix are generally hydrophilic gums such as Guar gum and Tragacanth of natural origin, Hydroxypropyl Methylcellulose (HPMC), Carboxymethyl cellulose (CMC) and Xanthan gum of semi-synthetic origin and polyacrylamide of synthetic origin. The release of drug from such initially dehydrated hydrogels involves simultaneous absorption of water (resulting in hydration, gelling and swelling of gum) and desorption of drug via a swelling controlled diffusion mechanism. As the gum swells and the drug diffuses out of it, the swollen mass, devoid of drug appears transparent or glasslike and therefore the system is sometimes called as glass hydrogel.

In such systems, the dissolution medium surrounding the controlled release device may enter the polymer at a rate that controls the drug release. The drug release follows the (case I) Fickain first order diffusion under equilibration condition. This case I transport is described by a diffusion coefficient where release mechanism of drug from swellable polymer matrix is governed by the diffusion process.

The fractional drug release by this mechanism from the slab is given by equation :

$$\frac{Mt}{M\infty} = 4\left[\frac{Dt}{\pi l^2}\right]^{1/2}$$

Where $\frac{Mt}{M\infty}$ = fraction drug released

D = Diffusion coefficient

l = Initial film thickness

t = Release time

The Fickian release indicates that the fractional drug release at any time is characterized by the constant multiplied by the square root of time.

Further, the release of drug through the swelling controlled release system taken by case II transport is described by a characteristic relaxation constant. The release kinetics is assumed to be controlled by a rate limiting relaxation phenomenon positioned at the advancing front which also follows first order kinetics:

$$\frac{Mt}{M\infty} = \frac{2K_o}{C_o l} t$$

The case II transport indicates that until the two penetration fronts meet, the fractional release at any time is linearly related to time.

Under certain conditions, zero order release can be achieved where the prevailing molecular mechanism is a coupling of diffusion and macromolecular relaxation as a result of which the drug diffuses outward with a kinetic behavior that is dependant on the relative ratio of diffusion and relaxation called as Anomalous behavior or non-Fickian behavior which is intermediate between Fickian and case II given by equation:

$$\frac{M_t}{M_\infty} = K_1 \sqrt{t} + K_2 t$$

The generalized expression for the previous equations is

$$\frac{M_t}{M_\infty} = Kt^n$$

where K (constant) is characteristic of the macromolecular network system and the drug , whereas diffusional exponent, n, is indicative of the transport mechanism. Fickain and case II release are defined by n equal to 0.50 and 1.00 respectively and Anomalous behavior is defined by value between 0.5 and 1.0.

2.1.2 Development criteria for design of OCRS

The basic considerations in designing of oral controlled release systems are :

a) Drug Candidate

b) Polymer system

c) Delivery system

2.1.2.1 Drug Candidate

The type of delivery system in oral controlled release dosage forms depends upon the physicochemical properties of the drug and its biopharmaceutics characteristics as given below :

a) Molecular weight of the drug: The lower the molecular weight, the faster and more complete the absorption. For drugs absorbed by pore transport mechanism, the molecular size threshold is 150 daltons for spherical compounds and 400 daltons for linear compounds. However, more than 95% of drugs are absorbed by passive diffusion. Diffusivity, defined as the ability of drug to diffuse through the membrane, is inversely related to the molecular size. The upper limit of drug molecular size is 600 daltons. Drugs with large molecular size are poor candidates for OCRS e.g. peptides and proteins.

b) Aqueous solubility of the drug: A drug with good aqueous solubility, especially if pH-independent, serves as a good candidate for controlled release dosage forms. Drugs with pH dependent aqueous solubility and drugs with poor aqueous solubility are not good candidate for OCRS. Absorption of poorly soluble drugs is dissolution rate-limited which means that the controlled release device does not control the absorption process; hence, they are poor candidates for such systems.

c) Apparent partition coefficient of the drug: Greater the apparent partition coefficient of a drug, greater is its rate and extent of absorption. Such drugs have increased tendency to cross even the more selective barriers like BBB. The apparent volume of distribution of such drugs also increases due to increased partitioning into the fatty tissues and since the blood flow rate to such tissues is always lower than that to an aqueous tissue like liver, they may exhibit characteristics of models having two or more compartments. The parameter is also important in determining the release rate of a drug from lipophillic matrix or device.

d) Drug pKa and ionization at physiologic pH: The pKa range for acidic drugs whose ionization is pH-sensitive is 3.0 to 7.5 and that for basic drugs is 7.0 to 11.0. For optimum

passive absorption, the drugs should be in non -ionized form at the site at least to an extent of 0.1 to 5%. Drugs existing largely in ionized forms are poor candidates for controlled delivery e.g. hexamethonium.

d) Drug stability: Drugs unstable in GI environment cannot be administered as oral controlled release formulation because of bioavailability problems e.g. nitroglycerine. A different route of administration should then be selected such as the transdermal route.

e) Mechanism and Site of Absorption: Drugs absorbed by carrier-mediated transport processes and those absorbed through a window are poor candidates for controlled release systems e.g. several B vitamins.

f) Biopharmaceutic Aspects of Route of Administration: For a drug to be successful as oral controlled release formulation, it must get absorbed through the entire length of G.I.T. Since the main limitation of this route is the transit time (a mean of 14 hours), the duration of action can be extended for 12 to 24 hours. The route is suitable for drugs given in dose as high as 1000 mg. A drug whose absorption is pH dependent, destabilized by GI fluids/enzymes, undergoes extensive presystemic metabolism (e.g. nitroglycerine), influenced by gut motility, has an absorption window and/or absorbed actively (e.g. riboflavin), is a poor candidate for oral controlled release formulation.

g)Absorption Rate: For a drug to be administered as controlled release formulation, its absorption must be efficient since the desired rate-limiting step is rate of drug release Kr i.e. Kr <<Ka. A drug with slow absorption is a poor candidate for such dosage forms since continuous release will result in a pool of unabsorbed drug e.g. iron. Aqueous soluble but poorly absorbed potent drugs like decamethonium are also unsuitable candidates since a slight variation in the absorption may precipitate potential toxicity.

h) Elimination Half-Life: Smaller the t1/2, larger the amount of drug to be incorporated in the controlled release dosage form. For drugs with $t_{1/2}$ less than 2 hours, a very large dose may be required to maintain the high release rate. Drugs with half-life in the range 2 to 4 hours make good candidates for such a system e.g. propranolol. Drugs with long half-life need not be

presented in such a formulation e.g. amlodipine. For some drugs e.g. MAO inhibitors, the duration of action is longer than that predicted by their half-lives. A candidate drug must have t ½ that can be correlated with its pharmacologic response.

i) Rate of Metabolism: A drug which is extensively metabolized is suitable for controlled release system as long as the rate of metabolism is not too rapid. The extent of metabolism should be identical and predictable when the drug is administered by different routes. A drug capable of inducing or inhibiting metabolism is a poor candidate for such a product since steady-state blood levels would be difficult to maintain.

j) Dosage Form Index (DI): It is defined as the ratio of $C_{ss.max}$ to $C_{ss.min}$. Since the goal of controlled release formulation is to improve therapy by reducing the dosage form index while maintaining the plasma drug levels within the therapeutic window, ideally its value should be as close to one as possible.

k) Therapeutic Range: A candidate drug for controlled delivery system should have a therapeutic range wide enough such that variations in the release rate do not result in a concentration beyond this level.

l) Therapeutic Index (TI): The release rate of a drug with narrow-therapeutic index should be such that the plasma concentration attained is within the therapeutically safe and effective range. This is necessary because such drugs have toxic concentration nearer to their therapeutic range. Precise control of release rate of a potent drug with narrow margin of safety is difficult. A drug with short half-life and narrow therapeutic index should be administered more frequently than twice a day. One must also consider the activity of drug metabolites since controlled delivery system controls only the release of parent drug but not its metabolite.

m) Plasma Concentration-Response Relationship: Drugs such as reserpine whose pharmacologic activity is independent of its concentration are poor candidates for controlled release systems.

2.1.2.2 Polymer system[12]

Since the required input in terms of time, money and manpower is tremendous in the development of a new drug molecule, the focus of pharma technology, is now mainly on modifying the delivery of the existing drugs. This effort is exemplified in the designing of sustained release dosage forms which aim at spatial as well as temporal delivery of drugs at a considerably lower cost. Polymers have played an important role in man's endeavor in development of such systems so it is very essential to study the various properties of polymers which in turn affect the release of drug from such formulations. These properties are as follows:

a) Porosity: Cross linking of polymeric chains affect the permeability. Higher degree of cross-linking reduces the permeability, when active agents are dispersed in polymers. The geometry plays an important role when one considers tortuosity, the pores present are assumed to form uniform cross connections. Hence the molecules have to travel a greater distance than if the pores were straight. In case of corrugation model, one assumes that there are places where the pores tend to be narrower. This geometry closely approximates porous polymers.

The arrangement of pores can also affect the release. The pores may be isolated or inter-connected. As porosity increases, the pores become inter-connected consequently increasing the availability of the active ingredient. For two dimensional matrix this increase is about 50-70% & for 3 dimensional it is about 20-43%.

Porosity can be achieved by variation in a fabrication procedure, relative leaching of soluble components (polymer blends or volatile diluents or plasticizer) or by careful micro-deformation of certain crystalline or glassy polymers. The swelling of polymer under applied environmental conditions increase porosity and hence release rate.

b) Hydrophillicity of the polymer: Hydrophilic polymers, which swell in presence of water, offer the best choice to modify the release rate of drug. Due to swelling, the distance to be traveled by the core drug before it encounters body fluid increases whereas the drug at the surface is immediately absorbed. This can be seen from table 2.3:

Table 2.3: Parameters affecting drug release from hydrophilic polymers

Drug	Polymer	Diff. Coeff. cm^2/sec	Partition coeff. polymer / water
Acetophenone	Polyethylene	3.5×10^{-8}	3.16
Chlormadinore acetate	Silicone rubber	3.03×10^{-7}	82
Estriol	Polyurethane ether	2×10^{-9}	133

c) Mobility of segments: Diffusion coefficient depends on mobility of polymer chain segments. Diffusion of a molecule in a polymer requires the co-operative movements of several polymeric chain segments. This is why the drugs have higher diffusion coefficient in polymers that have lower inter-chain forces such as silicone rubber and natural rubbers as compared with polystyrene. The local segmental mobility may be affected by chain interactions arising from hydrogen bonding, polar group interactions or simple Van der Waal's attraction. As the number of these grouping per unit chain segment length increases, the degree of interaction increases, the segmental mobility decreases and the permeation rate also decreases. These effects are especially pronounced in the case of symmetrical substitution of polar groups, since the packing of adjacent chain segment is somewhat facilitated leading to more effective interactions .

d) Nature of grafted polymer: The dependence of polymer membrane permeation properties on the nature of grafted polymer chain length, conformation and domain formation have been elucidated by using several membrane materials which were subjected to controlled graft co-polymerization procedure. The improved permeation barrier characteristics of poly (isoprene-g-methyl methacrylate) to inert gas reentrants were found for short chain or densified graft domains as compared with long chain or extended domains. It is also reported that the presence of short graft chains act as relatively inert fillers or excluded volume by chain packing effect, more effectively than does longer graft chains. The short chains are

considered to be distributed along the backbone chain allowing a more effective packing and structured densification than do the relatively isolated comparatively long chain domains.

e) Wettability: If liquid can wet (i.e. contact angle below 90°) the penetration takes place basically via capillary motion through pores. Pore size, volume, surface tension of liquid and its viscosity affect the penetration. If the liquid cannot wet the polymer (contact angle above 90°), release can take place only by dissolution of the drug particles or by permeation of the polymeric particles themselves. In this case, the basic factors controlling the diffusion of the liquid movement through the polymer are cross-linking, density and crystallinity of the polymer.

The pore volume may not be significantly affected with change in wettability. As wettability decreases, drug release rate decreases. This means that in the case of hydrophobic polymers (e.g. polyethylene) the pore network cannot be penetrated by capillarity, so that pore volume effectively available for drug diffusion is only that left by the already dissolved drug particles.

Table 2.4: Wettability and drug release rate

Polymer	Pore volume	Contact angle	Aspirin release rate mg/cm²/ sec
Acrylate	0.18	51° 01'	0.16
PVC-PTFE	0.12	100°01'	0.1
Polyethylene	0.13	95° 58'	0.02

For wettable polymers like acrylates, decrease in pore volume caused by any process like sintering, brings a corresponding decrease of the volume generated by capillarity and hence the volume available effectively for drug diffusion is reduced and drug release rate is slowed down.

Table 2.5: Effect of sintering time of polymer on release rate of drug

Sintering time	Pore volume m/g	Water penetration	Aspirin release rate
0	0.176	0.167	0.073
0.5	0.169	0.104	0.033
1-5	0.139	0.077	0.018

f) Excipients added during processing : During the processing of polymers, various excipients like fillers, plasticizers etc. are added to facilitate the polymerization process. These excipients can also alter the release rates of the drug. Plasticizers increase the permeability of polymers. Fillers, usually of inorganic origin, decrease the permeability. However, the effect is complicated by type, shape and amount of filler and its interaction with the polymer.

g) Crystallinity : Maximum interchain attraction, resulting in greatest mechanical strength, requires that the polymer chains be packed as densely as possible and that the polar groups of adjacent chains be in registry, so that there is an efficient geometric matching-up of interacting dipoles or hydrogen-bonding groups between the chains. As crystallinity increases, the segmental mobility of the polymeric chains decreases with subsequent reduction in the release rate.

h) Glass transition temperature: It is the temperature at which the phase transition of polymer from glassy to rubbery or vice-versa takes place. The lower the Tg, higher is the permeability for a given type of polymer. Rubbery polymers have high permeability but occasionally lack mechanical strength and must be reinforced by cross-linking. Lower the Tg, greater is the release of active ingredient.

i) Hydrophobicity of a polymer: The greater the uptake of water and the lower the Tg, of hydrophobic polymers, the greater is the release rate. The release from hydrophobic polymers also depends upon crystallinity, fillers, plasticizers, electrolyte addition etc.

2.1.2.3 Delivery system

The various systems utilized for the controlled delivery of drugs in the alimentary canal are already discussed in 2.1.

2.1.3 Application of OCRS to antibiotics

The major parameters used to quantify the effect of antimicrobial drugs are the minimum inhibitory concentration (MIC) and the minimal bactericidal concentration(MBC). Although these parameters are good predictors of the potency of the drug-microorganism interaction, they do not provide any information on the kinetics of drug action. For instance, the MBC value does not provide information on the rate of bactericidal activity and whether this rate can be enhanced by increasing antimicrobial concentrations. Similarly, the MIC does not provide any information about the persistent activity of the antimicrobial agent that remains following exposure to the drug.

Antibiotics can be divided into 3 categories based on their pharmacodynamic properties, including both their bactericidal activity and their persistent effects[13]:

2.1.3.1 Category I exhibits time dependent killing rather than concentration-dependent killing and produces short-term or persistent effect with most bacteria. The killing rate of these antibiotics saturates at concentrations of around 4-5 times the MBC. Thus, high concentrations will not kill bacteria faster than lower concentrations. Furthermore, bacterial regrowth starts soon after serum and tissue concentrations fall below MIC. Penicillins, cephalosporins and aztreonam exhibit this time course of antimicrobial activity.

In vitro studies of the pharmacodynamics of β-lactam antibiotics have shown that killing of bacteria, in particular gram-negative aerobic rods, is slow, time dependent and maximal at relatively low concentrations[14]. Further, elevation of the dose is not associated with increased bactericidal potency[15,16]. It has also been suggested that at concentrations much greater than the MIC, a paradoxical pattern may occur, i.e., a decrease in bacterial kill potency[17]. These findings have led to the hypothesis that continuously maintained concentrations above a

certain level, related to the MIC for the specific pathogen, would be more efficacious than the high-peak-and-trough concentrations obtained with an intermittent dosing regimen.

Efficacy studies in laboratory animals are in agreement with these *in vitro* findings. It was found that in order to obtain the same efficacy, the daily doses have to be 8-fold higher during intermittent infusion regimens than during continuous infusion[15]. Other studies have shown that the percentage of survival of the animals increased linearly with the frequency of dosing and time over the MIC but not with other pharmacodynamic parameters[18].

In addition to the pre-clinical findings, several clinical efficacy studies corroborate this concept, however, these are still scarce. Schentag et al. have shown a significant relationship between time to eradication of gram-negative pneumonia and time over MIC[19]. Weinstein et al. examined the relationship between β-lactam concentrations at trough and the success of therapy in patients with acute and chronic osteomyelitis[20].

It was found that maximum efficacy with β-lactam antibiotics in humans appeared to be dependent on maintaining levels above the MIC of the infecting organism for the majority of the dosage interval. The few randomized trials that have compared the efficacy of the β-lactams given by continuous vs. intermittent administrations also support this conclusion.

Exposures of staphylococci, streptococci, or enterococci to different β-lactams are consistently followed by post anti-biotic effects (PAEs) of several hours' duration[15].

This persistent suppression of gram-positive cocci growth enabled the development of intermittent dosing regimens for these drugs. Traditionally, intermittent intravenous infusions or intramuscular injections have been considered optimal dosing regimens that worked reasonably well in clinical practice. However, because of the increasing number of immuno-compromised patients, the rising incidence of gram-negative infections, and the availability of improved intravenous drug delivery systems, new strategies have been introduced for improving antimicrobial therapy with β-lactams. These strategies apply continuous infusions of these drugs to provide improved patient outcome with reduced doses of drug[16]. In summary, the goal of the dosage regimen of β-lactam drugs should be to prevent the drug-free interval doses from being long enough for the bacterial pathogen to resume growth.

2.1.3.1.1 Target therapeutic concentration[18]

Several investigators have proposed, based on *in vitro* experiments, that a maximum effect is reached at 4 x MIC for the target bacterium.

Preclinical investigators clarified that the target therapeutic concentration depends on the status of the immune system. For example, serum ceftazidime concentration needed during continuous infusion to obtain 50% efficacy in normal rats was between 1/6 and 1/5 the MIC, for the infecting K. pneumonia, depending on the severity of the infection. However, the concentration needed to obtain 100% efficacy (ED_{100}) was dependent not only on the severity of infection but also whether the animals were leukopenic or not.

2.1.3.1.2 Required time over MIC (T>MIC)[21,22,23]

Craig has summarized all the available data from the literature that use mortality as an endpoint and in which animals infected with *S . pneumoniae* were treated with penicillins or cephalosporins. The duration of time that the serum level needs to be above the MIC to ensure efficacy was determined in one study. Mortality was virtually 100% if serum levels were above MIC for 20% or less of the dosing interval. In contrast, as soon as T > MIC was 40-50% of the dosing intervals or higher, bacteriologic efficacy was 90-100%. Similar results were found in clinical studies that assessed bacteriologic cure in *Otitis media*. The findings indicate that if serum levels are above the MIC for 40-50% of the dosing interval, a bacteriologic cure of over 90% is obtained. In some β-lactum antibiotics, a T>MIC of 30% is also effective eg Amoxycillin.

The above approaches have been used to extend the activity of amoxycillin, a β-lactum, which has pharmacokinetic properties similar to other drugs from this category, including short elimination half-life and active absorption limited to the upper parts of the G.I. tract[24].

Prolongation of T>MIC for these drugs following oral administration is limited by the narrow absorption window. To overcome this pharmacokinetic limitation, the controlled release matrix tablet was designed, in the reported study, to release 50% of its content within 3 hours,

followed by a constant release rate for about 8 hours. The rapid onset of drug release was designed to provide an initial 'loading dose' and to maximize the absorption phase in those parts of the intestine in which amoxycillin is actively absorbed by a carrier-mediated process. The *in vivo* evaluation of this formulation revealed that the extent of absorption of the new formulation was not much different than that of a regular soft gelatin capsule formulation. Furthermore, the time required to obtain therapeutic concentration (onset time) was found to be identical for the two formulations. However, T>MIC and T>4 x MIC of the drug against susceptible pathogens was found to be maintained for a significantly longer period.

The following equation can be used to calculate T>MIC:

$$T>MIC(\%) = \frac{T>MIC \times 100}{\text{Dosing interval (Hrs.)}}$$

2.1.3.2 Category II is characterized by concentration dependent killing over a wide range of concentrations and by prolonged persistent effects. The higher the drug concentration, the greater the rate and extent of bacterial killing. This category includes aminoglycosides, fluoroquinolones and metronidazole. The 24hour AUC>MIC ratio is the parameter that best co-relates with the efficacy of fluoroquinolones. When tested for ciprofloxacin, this parameter was better than the peak drug concentration and considerably better than T>MIC needed to exceed the MIC for about 20% of the time interval to obtain any bacterial killing. This rationale was used for developing once a day formulation of Ciprofloxacin, vide US patent 6,261,601, by Ranbaxy Research Laboratories, India.

2.1.3.3 Category III contains drugs such as clindamycin and macrolides such as clarithromycin and azithromycin, which demonstrate minimal concentration dependent killing but have prolonged persisting effects. Abbot has launched once a day tablets of clarithromycin (Biaxin XL) which have been proved to provide equivalent drug absorption as compared to the original twice-daily formulation.

2.1.4 Use of Probenecid[25]

Many drugs and drug metabolites are actively secreted by the proximal tubular active transport mechanism and interactions may arise from competition for these systems. Particularly with antibiotic therapy, active tubular secretion is a significant route of elimination. Drugs that use the same active transport system in the kidney tubules can compete with one another for secretion. Probenecid may be used as an adjunct to antibacterial therapy particularly when treating severe or resistant infections. It reduces the tubular excretion of penicillins and most cephalosporins and may increase their plasma concentrations up to fourfold. The usual dosage for reducing tubular excretion of penicillins and cephalosporins is 500mg four times daily. Single doses of probenecid 1g are given together with an oral antibacterial or atleast 30minutes before an injected antibacterial, in single-dose treatment of gonorrhoea.

2.2 Requirements of PK studies[26,27]

Bioavailability is defined as the rate and extent to which the active ingredient is absorbed from a drug product and becomes available at the site of action. From a pharmacokinetic perspective, BA data for a given formulation provide an estimate of the relative fraction of the orally administered dose that is absorbed into the systemic circulation when compared to the BA data for a solution, suspension or intravenous dosage form. BA for orally administered drug products can be documented by developing a systemic exposure profile obtained from measuring the concentration of active ingredients over time in samples collected from the systemic circulation. Systemic exposure patterns reflect both release of the drug substance from the drug product and a series of possible presystemic/systemic actions on the drug substance after its release from the drug product. The regulatory objective is to assess, through appropriately designed BA studies, the performance of the formulations used in the clinical trials that provide evidence of safety and efficacy.

When the first modified-release drug product for a previously approved immediate-release product is prepared the purpose of an *in vivo* BA study is to determine if all of the following conditions are met:

- the drug product meets the controlled release claims made for it
- the BA profile established for the drug product rules out the occurrence of any dose dumping
- the drug product's steady-state performance is equivalent to a currently marketed noncontrolled release or controlled release drug product that contains the same active drug ingredient
- the drug product's formulation provides consistent pharmacokinetic performance between individual dosage units

The reference material for such a bioavailability study shall be chosen to permit an appropriate scientific evaluation of the controlled release claims made for the drug product such as:

-a solution or suspension of the active drug ingredient

-a currently marketed noncontrolled-release drug product containing the same active drug ingredient and administered according to the dosage recommendations in the labeling

-a currently marketed controlled-release drug product containing the same active drug ingredient and administered according to the dosage recommendations in the labeling

Bioequivalence (BE) is defined as the absence of a significant difference in the rate and extent to which the active ingredient in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

For a modified release product which is already marketed, only BE studies are required to be performed. Such BE studies should include:

- a single-dose nonreplicate, fasting study comparing the highest strength of the test and reference listed drug product

- a food-effect, nonreplicate study comparing the highest strength of the test and reference product.

Because single-dose studies are considered more sensitive in addressing the primary question of BE, multiple-dose studies are generally not recommended even in instances where nonlinear kinetics are present.

The following pharmacokinetic information is to be derived for BA/BE studies:

- plasma concentrations and time points
- subject, period, sequence, treatment
- $AUC_{0-\infty}$, C_{max}, T_{max}, λ and $t_{1/2}$

In addition the following statistical information should be provided for $AUC_{0-\infty}$ and C_{max}:

- Geometric mean
- Arithmetic mean
- Ratio of means
- Confidence intervals

Log transformation should be provided for measures used for BE demonstration.

2.3 Patent search

The relevant prior art methods, used for extending the release of some β-lactam antibiotics are as follows:

U.S. Pat. No. 4,250,166 discloses a long-acting cephalexin preparation comprising of normal quick-releasing cephalexin and particulate cephalexin coated with a copolymer of methylmethacrylate and methacrylic acid which dissolves at a pH from 5.5 to 6.5 and the potency ratio of the normal cephalexin to coated cephalexin is between 40:60 and 25:75.

U.S. Pat. No. 4,713,247 discloses a long-acting cefaclor formulation comprising of a mixture of non-enteric coated rapid-release cefaclor component and an enteric coated slow-release cefaclor component at a ratio of 4:6 based upon cefaclor potency, wherein the rapid-release

component releases the drug in gastric fluid while the slow-release component dissolves at pH 5 to 7, thereby enabling oral administration thereof twice a day.

U.S. Pat. No. 4,968,508 discloses a sustained release matrix tablet comprising from about 0.1 % to about 90 % by weight of cefaclor, about 5 % to about 29 % by weight of hydrophilic polymer and about 0.5 % to about 25 % by weight of an acrylic polymer which dissolves at a pH in the range of about 5.0 to about 7.4, the total weight of polymers being less than 30 % by weight of the formulation. Although a specific cefaclor formulation is claimed, the text suggests that the matrix formulation is suitable for weakly basic drugs and particularly suitable for cephalexin and cefaclor. A simulated gastrointestinal method has been used to evaluate the release profiles of the various compositions described. This method involves exposing the tablets for one hour to 0.1NHCl after which the pH in the dissolution kettle is increased to pH 6.8 by the addition of 250mL of 0.2M tribasic sodium phosphate. Some of the dissolution profiles obtained for 4-hours profile of cefaclor compositions, using the above method are disclosed in the patent as shown in Table 2.6 :

Table 2.6: Dissolution profiles of patented OCRS(BID) compositions

Time in minutes	Formula 1	Formula 2
30	17	24
60	34	44
90	56	59
120	71	68
180	88	87
240	100	99

U.S. Pat. No. 5,948,440 discloses a controlled release tablet of an active ingredient comprising of cefaclor, cephalexin, or their pharmaceutically acceptable hydrates, salts, or esters as active ingredient, and a mixture of hydrophilic polymers selected from the group consisting of at

least one hydroxypropyl methylcellulose and at least one hydroxypropylcellulose. The composition optionally also contains one or more of a water soluble or water dispersible diluent. The quantities of the hydrophilic polymers and water soluble or water dispersible diluent are such that the therapeutically effective active ingredient is released at a rate suitable for twice daily administration of the pharmaceutical composition. The patent also discloses the following dissolution method for *in-vitro* evaluation of the examples shown therein:

900mL of 0.1N HCl for one hour, after which the dissolution medium is changed to a pH 6.8 mixed phosphate buffer (900mL). Tablets are placed into a 40-mesh basket (USP Apparatus-Type –1) and rotated at 100rpm.

The dissolution profiles disclosed for compositions of cefaclor meant for BID application are shown in table 2.7:

Table 2.7: Dissolution profiles of patented OCRS(BID) compositions

Time in minutes	Formula 1	Formula 2
30	43.46	50.83
120	69.06	80.06
180	88.91	100.61
240	101.53	

U.S. Pat. No. 6,083,532 discloses a sustained release tablet comprising a drug to be released at a controlled rate and a sustained release formulation comprising at least three different types of polymers including a pH dependent gelling polymer, a pH independent gelling polymer and an enteric polymer wherein pH dependent gelling polymer comprises at least one of an alginate, a carboxyvinyl polymer, or a salt of a carboxymethyl cellulose; pH independent gelling polymer comprises at least one of a hydroxy propyl methyl cellulose, a hydroxy propyl ethyl cellulose, a hydroxy propyl cellulose, a hydroxy ethyl cellulose, a methyl cellulose, a

xanthan gum or a polyethylene oxide; and enteric polymer comprises at least one of a polyacrylate material, a cellulose acetate phthalate, a cellulose phthalate hydroxy propyl methyl ether, a polyvinyl acetate phthalate, a hydroxy propyl methyl cellulose acetate succinate, a cellulose acetate trimellitate, or a shellac.

U.S. Pat. No. 4,919,938 discloses a sustained release pharmaceutical composition in tablet form consisting essentially of a core matrix containing 20% to 60% by weight of a hydroxypropylmethylcellulose gelling agent, 0.41% to 20% by weight of (+)-trans-1a,2,3, 4a,5,6-hexahydro-9-hydroxy-4-(1-propyl)-4H-naphth [1,2-b]-1,4-oxazine hydrochloride, 2.08 to 12.5% by weight of buffering agent and suitable pharmaceutically acceptable excipients. A coating of a slowly soluble water permeable ethylcellulose polymer surrounds the core matrix.

U.S. Pat. No. 4,983,398 discloses a therapeutically active composition comprising a mixture of a therapeutically active medicament and a carrier base material, wherein the carrier base material consists essentially of one or more water-soluble, non-ionic cellulose ethers, wherein at least one of the cellulose ethers is a hydroxypropyl methylcellulose having a number average molecular weight of at least 50,000, and an alkali metal carboxylate. The carrier base comprises less than 30% by weight of the total weight of the composition.

U.S. Pat. No. 4,369,172 discloses a carrier base material combined with a therapeutically active medicament shaped and compressed to a solid unit dosage form having a regular and prolonged release pattern upon administration. The carrier base material is hydroxypropyl methylcellulose, or a mixture of hydroxypropyl methylcellulose and up to 30% by weight of a mixture of ethylcellulose and/or up to 30% by weight of the mixture of sodium carboxymethylcellulose, and wherein the hydroxypropyl methylcellulose has a hydroxypropyl content of 9-12% weight and a number average molecular weight of less than 50,000.

U.S. Pat. No.4,557,925 discloses a controlled release pharmaceutical tablet comprising a drug and a coating applied thereon. The coating comprises a film-forming forming polymer which is insoluble in water and gastrointestinal fluids and consists essentially of a terpolymer of polyvinylchloride, polyvinyl acetate and polyvinyl alcohol, and a water soluble pore creating material randomly distributed in the terpolymer coating. The pore creating substance is present in an amount of one part to 35 parts for each one to ten parts of terpolymer.

U.S. Pat. No. 4,726,951 discloses a pharmaceutical composition for oral administration with selectively adjustable programmed release and controlled absorption, comprising miniaturized granules obtained by high to very high compression. The pharmaceutical composition comprises miniaturized granules (a) containing pH control agents, (b) coated with excipients determining the slow penetration of digestive liquids, and/or (c) coated with a very thin layer of liquids or mixture of such granules, with the relative proportion of (a), (b) and (c) adjusted to give the desired release of the active ingredient. Cephalexin is one of the active ingredients disclosed.

U.S. Pat. No. 5,051,262 discloses a delayed action programmed release pharmaceutical preparation of one or more medicament units suitable for oral administration, each unit comprising an inert core surrounded by at least one inner layer and one or more inert outer coatings. At least one of the inner layers comprises an active medicament, which has a solubility which varies with pH and is either basic or acidic, and at least one pH adjuster. The pH adjuster is an organic acid or organic acid salt if the medicament is basic, or an inorganic base or basic salt if the medicament is acidic. The pH adjuster is present in an amount sufficient to ensure that the rate of dissolution of the medicament is substantially independent of the pH of the environment in which dissolution occurs. Cephalexin is described as one of the possible medicaments.

Patent Application WO 99/49868 discloses a sustained release cefaclor composition comprising 30 to 90 wt % of cefaclor, 5 to 60 wt % of a hydroswelling polymer and 1 to 10 wt % of a salt capable of releasing gaseous CO_2 in a gastric environment useful for administration once a day as well as twice a day. The amount of the salt added is critical as use of excessive amount of salt would generate excessive amount of CO_2 gas thereby irritating the stomach, disintegrating the formulation and losing the sustained release characteristic.

Japanese Patent JP 57165392A discloses a long-acting cephalexin tablet comprising cephalexin mixed with ≥10% w/w oils and fats (e.g. higher fatty acid, higher alcohol, alcohol ester, etc.) and with a vehicle such as microcrystalline cellulose and a lubricant such as magnesium stearate, and the mixture is pressed, formed to granules passing through a 20 mesh sieve, and subjected to the slug-forming process to obtain a high-quality long-acting tablet. The rate of dissolution of cephalexin can be controlled by selecting the kind of oils and fats and the number of the times of slug formation process.

Japanese Patent JP 07010758A discloses a long acting cefaclor composition comprising rapidly soluble cefaclor and a delayed soluble cefaclor prepared by enteric coating of hydroxypropyl methyl cellulose acetate succinate and triethyl citrate.

Patent application WO 98/22091 discloses a controlled release β-lactam antibiotic agent preferably amoxycillin trihydrate in a hydrophilic and/or hydrophobic polymeric matrix such that 50 % of the active is released within 3 to 4 hr from oral administration and remainder is released at a controlled rate. Examples include matrix tablets containing amoxycillin with hydroxypropyl methylcelluloses, amoxycillin with eudragit and alginate.

United States Patent 3,996,355 teaches permanent suspension dosage forms of water-sensitive drugs for administration without reconstitution. Amoxycillin-probenecid suspension dispersed in sesame oil containing sucrose as suspending agent and silica as thickening agent is exemplified.

Patent No. RO 80932 discloses oral suspension of benzathine penicillin, procaine penicillin and probenecid with other excipients.

Japanese Patent JP 52105220A discloses suppository formulations of β-lactams. For example, a suppository capsule containing cephalexin, probenecid, peanut oil and polyoxyethylene cetyl ether.

Japanese Patent JP 52064418A discloses highly absorbable penicillin suppository formulation containing (4-ethyl-2,3-dioxo-1-piperazinyl carbonyl amino)-benzyl penicillin or its salt, probenecid, and peanut oil.

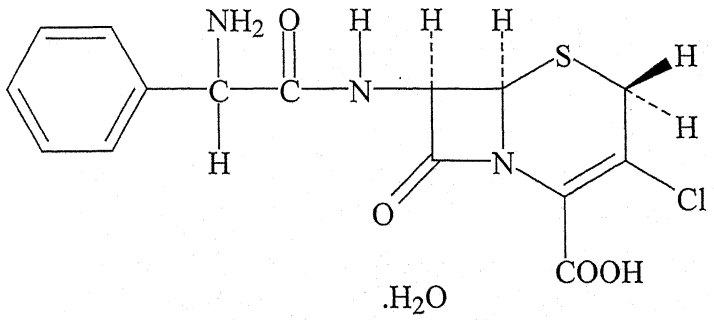
2.4 Drug substance

In this study, once a day formulation was to be developed for cefaclor in order to improve patient compliance and bring about life-cycle extension of the molecule.

Cefaclor is a semi synthetic, cephalosporin antibiotic for oral administration. Dosage forms of Cefaclor available in the market include capsules, dispersible tablets, extended release tablets, powder for oral suspension and redimix. Monographs of capsule, powder for oral suspension and extended release tablets are available in United States Pharmacopoeia and British Pharmacopoeia. Capsules are available in two strengths (250 & 500mg), dispersible tablets in two strengths (125 & 250mg), powder for oral suspension in three strengths (125,187 & 250mg/5ml), redimix in two strengths (125 7250mg/5ml) and extended release tablets in two strengths (375 & 500mg) for BID administration.

2.4.1 Physicochemical properties of drug substance[28]

Cefaclor is an orally active cephalosporin, which due to its greater activity against Gram-negative bacteria, particularly *Haemophilus influenzae,* is often classified as a second-generation agent. Cefaclor is a white to cream colored crystalline powder. The material is odorless going to slightly sulphurous. Chemically it is 3-chloro-7-d-(2-phenylglycinamide)-3-cephem-4-carboxylic acid, monohydrate. Its molecular weight is 385.82.



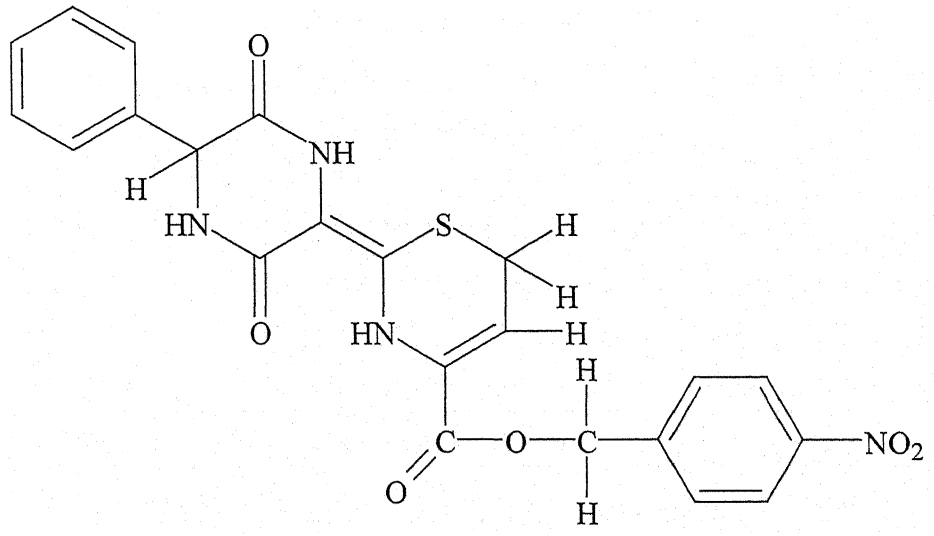
Polymorphs of cefaclor are possible. Such polymorphs are a function of the solvent from which cefaclor is crystallized. The only polymorph of general importance is cefaclor monohydrate. The X-ray powder diffraction data, as reported, for Cefaclor monohydrate is appended as Annexure 1.

The infrared spectrum of cefaclor monohydrate in potassium bromide pellet, as reported, is appended as Annexure 2.

The thermogram of cefaclor generally shows a small broad endotherm between 40°C and 120°C corresponding to the loss of water and other volatiles from the sample. The major endotherm in the DTA curve for cefaclor is observed around 220°C where the material decomposes. Cefaclor gives a reasonable thermo gravimetric curve and shows a loss of water and other volatiles from about 40°C to 120°C. At about 180°C, Cefaclor samples begin to lose weight indicating the beginning of decomposition of the sample.

Cefaclor is a reasonably stable molecule in the dry state. When cefaclor is present in the monohydrate crystalline form in the dry powder, two-year stability can be easily obtained. The powder becomes lightly yellow upon aging, however, little decrease in the potency of cefaclor is observed.

On degradation, cefaclor appears to lose HCl quite easily. Further degradation steps seem to be quite rapid and no other compounds have been isolated. In an attempt to generate such compounds, some studies have been carried out on the p-nitrobenzylester of cefaclor. This study showed that Cefaclor can undergo intramolecular nucleophilic attack by the side chain amine group to produce a diketopiperazine with the following structure:



Cefaclor is stable in solutions of pH not higher than 4.5. Solutions prepared in pH2.5 and 4.5 buffers contain at least 90% of their initial activity after 72 hours at 4°C. In neutral or alkaline solutions, cefaclor undergoes a rapid loss of activity.

The pharmacopoeial specification of Cefaclor is summarized in table 2.8 [29,30]:

Table 2.8: Pharmacopoeial specifications of Cefaclor

Test Parameter	Compendial limits	
	USP 25	**BP 2002**
Description	White to off white, crystalline powder. Slightly soluble in water; practically insoluble in methanol, in chloroform and in benzene.	A white or slightly yellow powder, slightly soluble in water, practically insoluble in methanol and in methylene chloride.
Identification	a) IR similar to USP Cefaclor RS b) The retention time of assay preparation of test preparation corresponds to that of USP Cefaclor RS	a) IR: Similar to cefaclor CRS b) By TLC: Similar to BP Cefaclor CRS c) Color development
Crystallinity	Meets the requirements	-
pH	Between 3.0-4.5, in an aqueous suspension containing 25mg/mL	Between 3.0-4.5, in an aqueous suspension containing 25mg/mL
Water (by KF)	Between 3.0-6.5 %	Between 3.0-6.5 %
Specific optical rotation	-	$+101^{\circ}$ to $+111^{\circ}$
Heavy metals	-	30ppm
Chromatographic purity Individual related substance Total related substances	NMT 0.5% NMT 2.0%	NMT 0.5% NMT 2.0%
Assay (HPLC)	950 to 1020µg of $C_{15}H_{14}ClN_3O_4S$ per mg on anhydrous basis.	NLT 96.0 and NMT the equivalent of 102.0% of $C_{15}H_{14}ClN_3O_4S$ on anhydrous basis.

2.4.2 Clinical pharmacology[31]

Cefaclor is the second generation Cephalosporin that is active by oral route having antibacterial activity.

2.4.2.1 Sensitive Organisms

Cefaclor is more active than Cephalexin against Gram-positive bacteria, such as staphylococci and streptococci, but against Staph. aureus it is not as active as cephalothin. The drug is somewhat less resistant to staphylococcal beta-lactamase than Cephalexin and so it may not be a reliable anti-staphylococcal agent[32,33].Enterococcus faecalis is resistant[34]. Cefaclor is more active than Cephalexin against many Gram-negative bacteria such as meningococci, gonococci, E.coli, Klebsiella pneumoniae , Pr. mirabilis and Salmonella and Shigellae spp.[35,36,37] The drug is active against beta-lactamase-producing gonococci[38]. Ampicillin-sensitive strains of H influenzae and most which are ampicillin – resistant because of beta-lactamase production are sensitive to Cefaclor. Most strains, which show intrinsic resistance to ampicillin, are Cefaclor resistant[39]. Moraxella catarrhalis is also usually Cefaclor-sensitive. Cefaclor is inactive against Serratia, Providencia and Acinetobacter spp. and Pseudomonas aeruginosa. Most strains, of Pr. Vulgaris and Morganella morganii are also resistant[40]. Anaerobic Gram-positive cocci and most Gram-negative anaerobes, other than those of Bacteroides fragilis group are usually cefaclor-sensitive. The Clostridium spp. are usually resistant[41].

The MICs of Cefaclor against some bacterial species are shown in table 2.9 [42-45].

Table 2.9: MICs of Cefaclor

Organism	MIC (μg/mL)
Gram-positive bacteria	
Staphylococcus aureus (non-penicillinase producer)	2.0
Staphylococcus aureus (penicillinase producer)	2.0
Streptococcus pyogenes (group A)	0.25
Enterococcus faecalis	64.0
Organism	**MIC (μg /mL)**
Gram-negative bacteria	
Escherichia coli	8.0
Enterobacter spp.	>128.0
Klebsiella spp.	8.0
Proteus mirabilis	>128.0
Proteus vulgaris	>128.0
Providencia spp.	>128.0
Serratia spp.	>128.0
Haemophilus influenzae	2.0

2.4.2.2 Mechanism of Action

Cefaclor inhibits bacterial septum and cell wall synthesis, probably by acylation of membrane bound transpeptidase enzymes. This prevents cross-linkage of peptidoglycan chains, which is necessary for bacterial cell wall strength and rigidity.

2.4.2.3 Mode of Administration and Dosage

Oral doses of 250-500 mg, 6 hourly, are suitable for adults[46]. An adult dosage of 0.5 g, 8-hourly is also satisfactory[47]. In children, the dosage is 40-50 mg per kg body weight per day, given in three or four divided doses[48]. For the treatment of milder infections in children, a dosage of 40 mg per kg, given in two divided doses, is also satisfactory[49].

Similar to amoxycillin and other drugs, Cefaclor in a dose of 2 g, can be used for single-dose treatment of acute uncomplicated urinary tract infections in adults[50].

Cefaclor's half-life in normal subjects of 40-60 min, only increases to 3 h in anephric patients[51].

As a result, its dose can be reduced in patients with renal failure to a lesser extent than cephalexin. Patients with severe renal failure should receive 25% of the usual dose, and those with moderate renal failure 50% of the usual dose. In patients with mild renal failure (creatinine clearance >40 mL per min), modification of Cefaclor dosage is unnecessary[52].

2.4.2.4 Serum Levels in Relation to Dosage

The drug is rapidly absorbed from the gastrointestinal tract, but its peak and subsequent serum levels are lower than with cephalexin. After a 200-mg oral dose, the mean peak serum level at 1 h is 6 μg per ml (comparable level for cephalexin 9.4), which falls to 0.3μg per mL (cephalexin 0.68) at 4h. Cefaclor is more rapidly excreted than cephalexin, their half –lives being 0.58 and 0.8 h, respectively[46]. Concomitant administration of Probenecid prolongs the serum levels of Cefaclor. Food intake reduces the maximum concentration of the Cefaclor in the serum and prolongs the time to attain this concentration. However, the area-under-the concentration-time curve and urinary recovery of the drug are unaffected[53]. The serum half-life of Cefaclor in patients with severe renal failure is only about 3 h, which suggests that it is also eliminated by non-renal mechanisms[54].

2.4.2.5 Toxicity

Therapy with this drug has been associated with a low frequency of side-effects. Gastrointestinal symptoms, such as diarrhea and nausea, have occurred in some 2.6% of treated patients. Cefaclor only has a minor effect on the anaerobic intestinal microflora.

Hypersensitivity phenomena, such as allergic rashes, have been noted in 1.55% of patients. Eosinophilia, positive Coombs' test without hemolysis, reversible leukopenia and elevated SGOT levels have also been noted occasionally. Serum sickness-like reactions appear to occur more commonly with Cefaclor than with cephalexin. These reactions occur with Cefaclor because of the drug' biotransformation in liver into immuogenic metabolites. An elevated

blood urea occurs occasionally during Cefaclor therapy, but serious nephrotoxicity has not been observed . Animal experiments show that unilateral obstruction of the ureter increases the nephrotoxicity of Cefaclor and of other Cephalosporins, which are rapidly secreted across renal tubular cells.

2.4.2.6 Clinical Uses of Cefaclor

This drug has been satisfactory for the treatment of urinary tract infections, including cases of complicated and/or recurrent infections. Pyelonephritis caused by ampicillin-resistant organisms, such as Klebsiella spp., also responds to Cefaclor. Uncomplicated urinary tract infections in non-pregnant women may respond to 2g single-dose Cefaclor therapy. A single daily dose of 250 mg cefaclor is satisfactory as prophylactic antibiotic for patients with recurrent urinary infections . Cefaclor has been curative for children and adults with acute streptococcal pharyngitis, otitis media and maxillary sinusitis. In children with acute otitis media it is about as good as amoxycillin. It is effective in otitis media and sinusitis caused by β-lactamase-producing strains of *H.influenzae and Moraxella catarrhalis* . Cefaclor is ineffective for eradicating *H.influezae* from pharyngeal carriers. It is about equally as effective as amoxycillin for the treatment of infective exacerbations of chronic bronchitis.

2.5 Polymers for preparing hydrophilic matrix

Xanthan gum and sodium alginate selected in the current study are high molecular weight biosynthetic polysaccharides and are extraordinarily enzymatically resistant. They offer potential utility as drug carriers because of their inertness and biocompatibility. A number of controlled drug release alginate systems have been studies such as gentamicin implants [55], pilocarpine ophthalmic film [56] and sulfadiazine tablets [57].

2.5.1 Sodium Alginate[58,59,60]

Alginic acid is a high molecular weight polysaccharide extracted from kelp(brown sea weed)and is neutralized with sodium carbonate to yield sodium alginate. Alginic acid is a linear copolymer of 1,4 linked β-D-mannuronic acid and α-L guluronic acid. The polymer chain consists essentially of three distinct polymer segments:

-Polymannuronic acid segments (M Blocks)

-Polyguluronic acid segments (G blocks)

-and segments of alternating or randomly distributed mannuronic acid and guluronic acid units (MG blocks)

The proportion of the 3 polymer segments varies between each species of kelp and imparts distinctly different properties to the final product. By altering the gulucuronate/manuronate ratio of the alginate, one can alter the gelling properties of the drug matrix and subsequent porosity of the gel. In turn, this will affect the rate of diffusion of the dissolved drug throughout the matrix. M-rich alginates are the most effective in sustaining the release of the drug from alginate powder tablets at acidic conditions. These are the most easily hydrating alginates at low pH and therefore, upon hydration, build up the diffusion and erosion barrier for the drug first. Recommended high M FMC products are Prontanal LF 240D and Protanal LF10/60D (60-70%M). Protanal LF 240D has a viscosity of 70-150mPa.s and particle size of 240-mesh. Keltone HVCR of ISP Alginates, has a medium M/G range , particle size of 80-mesh and viscosity of 400 mPa.s for a 1% dispersion.

Standards have been set forth for Sodium Alginate in the United States Pharmacopeia/National Formulary, the European Pharmacopoeia, British Pharmacopoeia, the Japanese Excipients Book and Food Chemicals Codex. Sodium alginate is considered Generally Recognized as Safe (GRAS) by qualified experts and is in accordance with United States Food and Drug Regulations.

Sodium alginate can be processed by direct compression, non aqueous granulation and aqueous granulation processes.

Sodium alginate, being the water-soluble salt of alginic acid, is insoluble below pH 3.0 and soluble above pH 3.0. This pH-dependent behavior of alginate can be exploited to customize release profiles. The matrix formed by sodium alginate releases the drug slowly below pH3.0 and shows faster release rate above pH 3.0. When ingested, the gastric fluid promotes a polymer chain relaxation forming a swollen gel layer of nearly infinite viscosity around the tablet. The hydrated sodium alginate is converted into a porous, insoluble alginic acid skin.

Once passed into the higher pH of the intestinal tract, the alginic acid skin is converted to a soluble viscous layer. This polymer matrix later begins eroding from the tablet surface into the gastro-intestinal fluid. Drug dissolution is dependent on both diffusion and erosion of tablet. Relative proportions of the released drug are determined by drug characteristics and the physiochemical nature of the gel layer.

Alginate salts can be used alone or in combination with other polymers such as xanthan gum to control drug release from hydrophilic matrix tablet.

One of the most important and useful properties of alginates is the ability to form gels by reaction with calcium salts[61,62]. These gels, which resemble a solid in retaining their shape and resisting stress, consist of almost 100% water. The cross links are proposed to have been caused either by simple ionic bridging of two carboxyl groups on the adjacent polymer chains via calcium ions or by chelation of single calcium ions by hydroxyl and carboxyl groups on each of a pair of polymer chains. Although these bonds may play a role in the gelation mechanism, they are not sufficiently energetically favorable to account for the gelation of alginate. It has been shown on the basis of fiber diffraction data and model building calculations that the shape of both polymannuronic acid segments and the polyguluronic acid segments of alginic acid is ribbon-like and extended and that these extended ribbons can stack together in sheets. On the basis of these data and the properties of gels, it has been suggested that the cooperative association of either polymannuronic acid segments or polyguluronic acid segments is involved in the formation of the cross linked network of polymer chains.

2.5.2 Xanthan gum

Xanthan gum is a high molecular weight natural carbohydrate produced in a pure culture fermentation process by the Xanthomonas campestris microorganism. In the fermentation process, Xanthomonas campestris is cultured in a well-aerated medium containing glucose, a suitable nitrogen source, di-potassium hydrogen phosphate and trace elements. To provide seed for the final fermentation, the microorganism is grown in several stages with associated

identification tests prior to introduction into the final fermentation medium. At the conclusion of the fermentation process, xanthan gum is recovered by precipitation in isopropyl alcohol and is then dried and milled. Xanthan gum is less prone to natural variation unlike naturally occurring gums. It is of unvarying chemical structure and has uniform chemical and physical properties.

Each xanthan gum repeat unit contains five sugar residues: two glucose; two mannose, and one glucuronic acid. The polymer backbone consists of four β-D –glucose units linked at the 1 and 4 positions and is therefore identical in structure to cellulose. Trisaccharide side chains on alternating anhydro-glucose units distinguish xanthan from cellulose. Each side chain comprises a glucuronic acid residue between two mannose units. At most of the terminal mannose units is a pyruvate moiety; the mannose nearest the main chain carries a single group at C-6. The resulting stiff polymer chain may exist in solution, as a single, double or triple helix which interacts with other xanthan gum molecules to form complex, loosely bound networks[63,64].

Xanthan gum is an anionic material and is not usually compatible with cationic surfactants, polymers and preservatives since precipitation occurs. Under highly alkaline conditions polyvalent metal ions, such as calcium, cause gelation or precipitation. Xanthan gum solutions are stable in the presence of up to 60% water miscible organic solvents such as acetone, methanol, ethanol or propanol. However, above this concentration precipitation or gelation occurs[65].

When formulation comprising a sustained release carrier comprising a major proportion of xanthan gum, and the pharmacologically active ingredient comes into contact with an aqueous medium, the xanthan gum in the portion of the formulation exposed to the aqueous medium hydrates and swells to form a gel. Xanthan gum has a good swelling action on contact with an aqueous medium and overcomes the problems encountered by gums which either do not hydrate rapidly enough or hydrate too rapidly. Gums which do not readily hydrate are generally unable to hold the tablet together as, on exposure to an aqueous medium, the tablet

tends to break up before the gel fully hydrates. Gums which hydrate too rapidly generally also break up quickly as the gel formed is usually very weak and is unable to hold the tablet together. The thickness of the gel surrounding the central core of composition is intermediate between that of the thin layer when a hard gel is formed, as formed by Hydroxypropyl Methylcellulose gels for example and the thick layer when a soft gel is formed. In addition the nature of the gel formed is such that unlike hard gels it may be readily deformed, unlike soft gels it is not disrupted by such deformation and *in-vivo* it may be expected to pass obstructions and not be impeded in the gastro-intestinal tract.

Gelling of xanthan gum is temperature independent, pH independent and allows the active ingredient to diffuse out of the formulation at a steady rate as the medicament passes through the digestive system irrespective of the pH. Thus any formulation containing it, is adapted to provide sustained release both in the acidic media of the stomach and also in the intestines.

The use of xanthan gum in the sustained release carrier generally allows a slower release of active ingredient into the body as compared to the use of naturally occurring hydrophilic gums. As a result, this provides the advantage that the proportion of sustained release carrier in the formulation may be reduced compared to most other sustained release formulations, thus enabling the sustained release formulation to be provided in a relatively small solid dosage form if desired[66].

2.5.3 Hydroxypropyl Methylcellulose Phthalate

It is prepared by the esterification of hydroxypropyl methylcellulose with phthalic anhydride. Several different types of HPMCP are commercially available with molecular weights in the range of 20000-200000. It is used alone or in combination with other soluble or insoluble binders in the preparation of granules with sustained drug release properties; the release rate is pH dependent. When so used, it is dissolved in either dichlormethane:ethanol or ethanol:water solvent mixture[67,68].

2.6 Drug product

2.6.1 Reference Listed Drug[69]

BID formulation of cefaclor is marketed by Eli Lilly under the brand name of CECLOR CD in USA. It is available in 2 strengths: 375mg and 500mg. The details of this product as described in Physician's Desk Reference are as follows:

2.6.1.1 Composition

Each Ceclor CD tablet contains Cefaclor monohydrate equivalent to 375 mg (1.02 mmol) or 500 mg (1.36 mmol) anhydrous Cefaclor.

In addition, each extended release tablet contains the following inactive ingredients: cellulose; FD&C Blue No.2; magnesium stearate; mannitol; methacrylic acid copolymer type C; propylene glycol; Stearic acid; titanium dioxide; polyethylene glycol; talc; and edible black ink.

2.6.1.2 Pharmacokinetics: The Ceclor CD formulation of Cefaclor is pharmacokinetically different from Ceclor® Pulvules® (conventional tablets of Cefaclor) formulation as can be seen from Table 2.10:

Table 2.10: Comparative pharmacokinetics of ceclor pulvules Vs ceclor CD in fasting and fed states

Parameter	**Ceclor CD**		**Ceclor CD**		**Ceclor Pulvules**	
	375 mg		**500 mg**		**2 x 250 mg**	
	fed	fasted	fed	fasted	fed	fasted
	n=10		n=16	n=16	n=15	n =16
C_{max}	3.7 (1.1)	NA	8.2 (4.2)	5.4 (1.6)	9.3 (2.7)	16.8 (4.7)
T_{max}	2.7 (1.0)	NA	2.5 (0.8)	1.5 (0.7)	1.5(0.6)	0.9 (0.4)
AUC	9.9 (2.2)	NA	18.1(4.2)	14.8 (4.0)	20.5 (2.8)	19.2 (5.0)

(± 1 standard deviation)

NA = data not available

No direct comparisons with the suspension formulation of Cefaclor have been conducted; therefore, there are no data with which to compare the suspension formulation. Until further data are available, the pharmacokinetic equivalence of the CD and the suspension formulations should NOT be assumed.

Absorption and Metabolism: The extent of absorption (AUC) and the maximum plasma concentration (C_{max}) of Cefaclor from Ceclor CD are greater when the extended release tablet is taken with food. No drug accumulation was noted when Ceclor CD was give twice daily. The plasma half-life in healthy subjects is independent of dosage form and averages approximately 1 hour.

Food Effect on Pharmacokinetics : When Ceclor CD is taken with food, the AUC is 10% lower while the Cmax is 12% lower and occurs 1 hour later compared to Ceclor Pulvules. In contrast; when Ceclor CD is taken without food, the AUC is 23% lower while the C_{max} is 67% lower and occurs 0.6 hours later using an equivalent milligram dose of Ceclor Pulvules as a reference. Therefore, Ceclor CD should be taken with food.

2.6.1.3 Indications and usage

When administered at the recommended dosages and durations of therapy, Ceclor CD is indicated for the treatment of patients with the following mild to moderate infections when caused by susceptible strains of the designated organisms.

-acute bacterial exacerbations of chronic bronchitis due to Haemophilus influenzae (non-β-lactamases-producing strains only), Moraxella cararrhalis (including β-lactamases-producing strains) or Streptococcus pneumoniae. (In view of the insufficient numbers of isolates of β-lactamases-producing strains of Haemophilus influenza that were obtained from clinical trials with Ceclor CD for patients with acute bacterial exacerbations of chronic bronchitis or secondary bacterial infections of acute bronchitis, it was not possible to adequately evaluate the effectiveness of Ceclor CD for bronchitis known, suspected or considered potentially to be caused by β-lactamase-producing H-influnezae.)

-secondary bacterial infections of acute bronchitis due to Haemophilus influenzae (non-β-lactamase-producing strains), or Streptococcus pneumoniae.

-pharyngitis and tonsillitis due to Streptococcus pyogenes.

-uncomplicated skin and skin and structure infections due to Staphylococcus pyogenes that were obtained from clinical trials with Ceclor CD for patients with uncomplicated skin and skin structure infections, it was not possible to adequately evaluate effectiveness of Ceclor CD for skin infections known, suspected, or considered potentially to be caused by S.pyogenes.

The safety and effectiveness of Ceclor CD in treating some of the indications and pathogens for which other formulations of Cefaclor are approved have NOT been established.

2.6.1.4 Contraindications

Ceclor CD is contraindicated in patients with known hypersensitivity to Cefaclor and other Cephalosporins.

2.6.1.5 Precautions

General: Superinfection (overgrowth by non susceptible organisms) should always be considered a possibility in a patient being treated with a broad-spectrum antimicrobial. Careful observation of the patient is essential. If superinfection occurs during therapy, appropriate measures should be taken.

Drug Interactions:

Antacids- the extent of absorption of Ceclor CD is diminished if magnesium or aluminum hydroxide-containing ant-acids are taken within 1 hour of administration; H2 blockers do not alter either the rate or the extend of absorption of Ceclor CD.

Probenecid- The renal excretion of Cefaclor is inhibited by Probenecid.

Warfarin- there have been rare reports of increased prothrombin time with or without clinical bleeding in patients receiving Cefaclor and warfarin concomitantly. No specific studies have been performed to rule in or rule out this potential drug/drug interaction.

Laboratory Test Interactions: Administration of Ceclor CD may result in a false-positive reaction for glucose in the urine. This phenomenon has been seen in patients taking Cephalosporin antibiotics when the test is performed using Benedict's and Fehling's solutions and also with Clinitest®tablets.

Labor and Delivery: Ceclor CD has not been studied for use during labor and delivery. Treatment should be given only if clearly needed.

Nursing Mothers: No studies in lactating women have been performed with Ceclor CD. Small amounts of Cefaclor ($\leq$ 0.21 μg/mL) have been detected in human milk following administration of single 500-mg doses of Ceclor. The effect on nursing infants is not known. Caution should be exercised when Ceclor CD is administered to a nursing women.

Pediatric Use: Safety and effectiveness of Ceclor CD in pediatric patients less than 16 years of age have not been established.

Geriatric Use : Healthy geriatric volunteers (≥ 65 years old) who received a single 750-mg dose of Ceclor CD had 40%-50% higher AUC and 20% lower renal clearance values when compared to healthy adult volunteers less than 45 years of age. These differences are considered to be primarily a result of age-related decreases in renal function. In clinical studies when geriatric patients received the usual recommended adult doses, clinical efficacy and safety were comparable to results in non-geriatric adult patients. No dosage changes are recommended for healthy geriatric patients.

2.6.1.5 Adverse reactions

Clinical Trials : There were 3272 patients treated with multiple doses of Ceclor CD in controlled clinical trials and an additional 211 subjects in pharmacology studies. There were no deaths in these trials thought to be related to toxicity from Ceclor CD. Treatment was discontinued in 1.7% of patients due to adverse events thought to be possibly or probably drug-related.

The following adverse clinical and laboratory events were reported during the Ceclor CD clinical trials conducted in North America at doses of 375 mg or 500 mg BID; however, relatedness of the adverse events to the drug was not assigned by clinical investigations during the trials. Adverse reactions occurring during the clinical trials with Cefaclor extended release tablets with an incidence of less than 1% but greater than 0.1% included the following (listed alphabetically):

Accidental injury, anorexia, anxiety, arthralgia, asthma, bronchitis, chest pain, chills, congestive heart failure, conjunctivitis, constipation, dizziness, dysmenorrhea, dyspepsia, dysuria, ear pain, edema, fever, flatulence, flu syndrome, gastritis, infection insomnia, leucorrhoea, lung disorder, maculpapular rash, malaise, menstrual disorder, myalgia, nausea and vomiting, neck pain, nervousness, nocturia, otitis media, pain, palpitation , peripheral edema, rash, respiratory disorder, sinusitis, somnolence, sweating, tremor urticaria, vomiting.

One case of serum-sickness-like reaction was reported among the 3272 adult patients treated with Ceclor have also been reported with the use of Cefaclor in other oral formulation and are

seen more frequently in pediatric patients than in adults. These reactions are characterized by findings of erythema multiforme, rash, and other skin manifestations accompanied by arthritis/arthralgia, with or without fever, and differ from classic serum sickness in that there is infrequently associated lymphadenopathy and proteinuria, no circulating immune complexes and no evidence to date of sequelae of the reaction. Such reactions have been reported with overall occurrence ranging from 1 in 200 (0.5%) in one focused trial; to 2 in 8346(0.024%) in overall clinical trials (with an incidence in pediatric patients in all clinical trials of 0.055%); to 1 in 38,000 (0.003%) in spontaneous event reports. Signs and symptoms usually occur a few days after initiation of therapy and subside within a few days after cessation of therapy. Occasionally these reaction have resulted in hospitalization, usually of short duration (median hospitalization = 2 to 3 days, based on postmarketing surveillance studies). In those patients requiring hospitalization, the symptoms have ranged from mild to severe at the time of admission with more of the severe reactions occurring in pediatric patients.

In Postmarketing Experience : In addition to the events reported during clinical trials with Ceclor CD, the following adverse experiences are among those that have been reported during worldwide postmarketing surveillance:allergic reaction, anaphylactoid reaction, angiodema, face edema, hypotension, Stevens-Johnson syndrome, syncope, paresthesia, vasodilatation, and vertigo.

Clinical: Severe hypersensitivity reactions, including Stevens-Johnson syndrome, toxic epidermal necrolysis, and anaphylaxis, have been reported rarely. Anaphylactoid events may be manifested by solitary symptoms, including angioedema, edema (including face and limbs), parasthesias, syncope, or vasodilatation. Anaphylaxis may be more common in patients with a history of penicillin allergy. Rarely, hypersensitivity symptoms may persist for several months.

2.6.1.6 Dosage and administration

The absorption of Ceclor CD is enhanced when it is administered with food. Therefore, Ceclor CD should be administered with meals (i.e., at least within one hour of eating). The extended release tablets should not be cut, crushed, or chewed. The dosage is as shown in the following Table 2.11:

Table 2.11: Dosage regimen of Cefaclor CD

Adults (age 16 years and older)	**Total Daily Dose**	**Dose and Frequency**	**Duration**
Acute Bacterial Exacerbations of Chronic Bronchitis due to H.influenzae (non-β-lactamase-producing strains), or Streptococcus pneumoniae	1000 mg	500 mg q 12 hours	7 days
Secondary Bacterial Infections of Acute Bronchitis due to H.influenzae (non-β-lactamase-producing strains only), M,catarrhalis (including β-lactamase-producing strains), or S.pneumoniae	1000 mg	500 mg q 12 hours	7 days
Pharyngitis and / or tonsillitis due to S.pyogenes	750 mg	375 mg q 12 hours	10 days
Uncomplicated Skin and Skin Structure infections due to S.aureus (methicillin-susceptible strains)	750 mg	375 mg q 12 hours	7 – 10 days

500 mg BID of Ceclor CD is clinically equivalent to 250 mg TID of Cefaclor as a pulvule in those indications listed in the section 2.4.1.3. 500 mg BID of Ceclor CD is NOT equivalent to 500 mg TID of other Cefaclor formulations.

Elderly patients with normal renal function do not require dosage adjustment.

2.6.1.7 Clinical studies

(i) In adequate and well-controlled clinical trials of Ceclor CD in the treatment of acute bacterial exacerbations of chronic bronchitis (ABECB) and secondary bacterial infection of acute bronchitis (SBIAB), only 4 evaluable patients with SBIAB had infections caused by β-lactamase-producing *H.influenzae*. Four patients do not provide adequate data upon which to judge clinical efficacy of Ceclor CD against β-lactamase-producing *H.influenzae*.

(ii) Ceclor CD (375 mg Q12H) (n=115) was compared to Ceclor Pulvules (250 mg TID) (n =106) for the treatment of patients with uncomplicated skin and skin structure infections, including cellulites, pyoderma, abscess, and impetigo. Patients were treated for 7 to 10 days and were evaluated for clinical resolution and bacterial eradication approximately one week after completing therapy. To be evaluable, all patients had to have a recognized pathogen isolated from the skin infection just prior to the initiation of therapy. The results of this randomized, double-blinded, U.S. trial demonstrated:

a) overall clinical cure rates were 72% (83 of 115 patients) and 75% (80 of 106 patients), respectively for Ceclor CD and Ceclor Pulvules (95% CI around 3% difference=-16% to + 9%),

b) overall bacteriologic eradication rates against Straphylococcus aureus were comparable

2.6.1.8 How supplied

Tablet (extended release):

375 mg, blue (UC 5391)- (60s)

500 mg, blue (UC 53492)-(60s)

500 mg, blue (UC5392) –(14s)

2.6.1.9 Storage condition

Store at controlled room temperature , 15° to 30°C (59° to 86°F).

2.6.2 Pharmacopoeial requirements for extended release tablets of Cefaclor

The compendial monograph for Cefaclor extended release tablets is available in USP and BP. The specifications for the drug product are shown in the following table 2.12 [70,71]:

Table 2.12: Pharmacopoeial requirements for Cefaclor extended release tablets

Test Parameter	Compendial limits	
	USP 25	**BP 2001**
Identification	Retention time of major peak in chromatogram of assay preparation correspond to that in the chromatogram of standard preparation as obtained in assay.	a)UV b)Retention time of major peak in chromatogram of assay preparation correspond to that in the chromatogram of standard preparation as obtained in assay.
Dissolution	Medium 0.1 N HCl, 900 mL Apparatus 1: 100 rpm The percentage of the label claim of Cefaclor dissolved at specified times conform the limit: Time (Min) — Amount dissolved 30 — 5 - 30 % 60 — 20 – 50 % 240 — NLT 80.0 %	A suitable dissolution test is carried out to demonstrate the appropriate release of Cefaclor. The dissolution profile reflects the *in vivo* performance, which in turn is compatible with dosage schedule, recommended by manufacturer.
Uniformity of dosage units	Meets the requirement	-
Water	NMT 7.0%	-
Assay	Cefaclor tablets contain the equi. of NLT 90.0% and NMT 110.0% of the labeled amount of $C_{15}H_{14}ClN_3O_4S$	90.0-105.0% of the stated amount of anhydrous cefaclor
Packaging and storage	Preserve in tight, light resistant containers	Store at a temperature not exceeding 30°C

2.7 Conclusions from literature surveyed

- Cefaclor exhibits pH dependent aqueous solubility.
- On account of sensitivity of cephalosporins to moisture and heat, a manufacturing process based on dry granulation or non aqueous wet granulation would improve stability of the drug product.
- Cefaclor has a short half life and hence to maintain the concentration above MIC, QID/BID dosing is required for immediate release products. Hence it is an ideal candidate for development of "dose dependent" to "rate controlled" extended release formulation in order to improve patient convenience and compliance.
- Although a number of patents exist for the BID formulation of cefaclor, none exist for OD formulation.
- The patents for preparing BID matrix formulations revealed the use of various cellulose polymers like Hydroxypropyl Methylcellulose, Ethyl cellulose, Hydroxypropyl cellulose, Hydroxyethyl cellulose, Methylcellulose besides Acrylic acid polymers, Xanthan gum, Sodium alginate, Cellulose acetate phthalate and Shellac.
- The major disadvantage of using the cellulose polymers is that they are required to be used in higher concentrations and with high dose high frequency drugs, the matrix formulation becomes too big for human consumption.
- Xanthan gum, has pH independent swelling characteristics and swells considerably at very low concentrations. Thus it is required to be added in relatively lower concentrations than most sustaining polymers and hence is suitable for extended release products of high dose high frequency drugs.
- Sodium alginate, shows a pH dependent release profile, with a slow release below pH 3.0 and faster one above pH3.0. This behavior can be exploited to customize release profiles.
- Sodium alginate gels can be stabilized by addition of Calcium ions.
- Alginate salts can be used alone or in combination with other polymers such as xanthan gum to control drug release from hydrophilic matrix tablet.

- Cefaclor absorption is restricted to proximal part of gastrointestinal tract. Hence the polymer system used in the matrix should contain a mixture of pH-dependent and pH independent polymers so as to reduce the effect of pH on the release rate of the drug substance.
- Based on the dissolution method and profiles mentioned in various patents for the BID product, the following method and limits could be adopted during formulation development of this product:

  Dissolution medium:

  0.1N HCl: for the first hour

  pH 6.8 Phosphate buffer: from 1 hour to 4 hours

  Apparatus: USP Type 1, 100rpm

  Limits:

Time	% released
1 hr	20-50%
2 hr	40-60%
3 hr	60-90%
4 hr	NLT 85%

  For the OD product, the following method could be adopted during formulation development:

  Dissolution medium:

  0.1N HCl: for the two hour

  pH 6.8 Phosphate buffer: after the second hour

  Apparatus: USP Type 1, 100rpm

  The dissolution profile for the OD product can be decided based on the BID product's *in-vitro- in-vivo* correlation.

- For cefaclor, as for all β-lactum antibiotics, elevation of the drug concentration above a critical value, which tends to be the minimal inhibitory concentration (MIC), is not associated with increased bacteriocidal potency. High concentrations are associated with reduced potency.

- There is a direct correlation between the time above MIC (T>MIC) and antimicrobial potency with 90% bacteriological cure being effected if T>MIC is greater than 40%.

- Cefaclor follows linear pharmacokinetics. Thus increasing the dose increases AUC linearly. In extended release systems, the rate of absorption is governed by release rate of drug from the dosage form, which can lead to reduction in AUC and hence efficacy. To avoid this, it is important to ensure that the AUC achieved by the extended release system is comparable to that achieved by an equal dose of immediate release dosage form.
- The excretion of cefaclor is reduced when given concurrently with probenecid resulting in increased and prolonged antibiotic serum concentration and prolonged half life. Hence co-administration of probenecid with a lower dose of cefaclor extended release formulation could prolong the T>MIC besides resulting in a comparable AUC.
- The absorption of Cefaclor is affected by food. Hence bioavailability/bioequivalence study must be performed in fed volunteers.

Based on the above literature findings, it was evident that there is a need for a simple, easy to make and cost effective cefaclor composition, which can be administered twice/once daily. The study objectives were redefined as follows:

i) To design a OCRS-BID of Cefaclor such that 90% confidence interval for the Cmax, AUC $_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ is in line with USFDA/EMEA guidelines with respect to Ceclor CD, 500mg.

ii) To design a OCRS-OD containing 1000/1500mg of Cefaclor, with or without Probenecid, such that the ratio of test to reference for C_{max} and $AUC_{0\text{-}\infty}$ is in line with USFDA/EMEA guidelines with respect to 3 capsules of 500mg of the conventional product, the T>MIC(1μg/mL)is greater than 40% of the dosing interval and $t_{1/2}$ is prolonged.

3

EXPERIMENTAL-I

CEFACLOR OCRS-BID

3.1 Characterization of drug substance

The drug substance used in the study was tested as per pharmacopoeial and in-house tests to evaluate its physicochemical properties. The results of these tests are summarized in Table 3.1:

Table 3.1: Results of drug substance characterization

Test Parameter/Batch	**CFC/IV/037/95** Mfg: 10/95	**CFCM049028** Mfg: 12/99
Description	White to off white, crystalline powder. Slightly soluble in water, practically insoluble in methanol, in chloroform and in benzene.	White to off white, crystalline powder. Slightly soluble in water, practically insoluble in methanol, in chloroform and in benzene.
Identification	Confirms BP	Confirms BP
Crystallinity	Meets the requirements	Meets the requirements
pH	4.0	3.7
Water (by KF)(%)	6.4	4.7
Specific optical rotation	114.70°	Not reported
Chromatographic purity Highest Individual Total related substances	 0.162% 0.50%	 <0.5% 0.22%
Assay (on anhydrous basis)	986µg/mg	991µg/mg
Bulk density(g/mL) Untapped Tapped	 0.45 0.64	 0.46 0.62
Particle size distribution (By sieve analysis) # size	% Retained	
#40	Nil	Nil
#60	3.2	2.4
#100	16.7	11.7
#200	43.4	34.6
Pan	36.7	51.3

3.2 Attributes of extended release reference listed drug products:

Cefaclor extended release tablets (375 & 500mg) are available in the US market as Ceclor CD® 375 & 500 mg tablets for BID dosage regime. Some important attributes of these products, reported or studied in the laboratory are shown in Table 3.2 :

Table 3.2: Results of reference listed drug product analysis

Sr. No	Parameters	375 mg	500mg
		CECLOR CD	CECLOR CD
1	Description	Blue colored, biconvex, film coated tablet printed with CECLOR CD 375 MG on one side.	Blue colored, biconvex, film coated tablet printed with CECLOR CD 500 mg on one side.
2	Inactive Ingredients	Hypromellose, Magnesium Stearate, mannitol methacrylic acid, co-polymer type C, propylene glycol, Talc and color mixture dark blue YS-1-4273.	Celluloses, FD& C blue no 2, Magnesium stearate, mannitol methacrylic acid, co-polymer type C, propylene glycol, Stearic acid, titanium dioxide, Polyethylene glycol, Talc.
3	Average weight	500 mg	700 mg
4	Dissolution profile	% drug release	
	1 hr	29.7	28.8
	2 hr	66.9	60.7
	3 hr	94.6	82.0
	4 hr	97.4	92.7
5	Storage condition	Store below 25° C	Store at room temperature (15-30°C).
6	Shelf life	2 years	2 years

3.3 Selection of excipients

Excipeints used in Lab scale trials were either sourced locally or imported and checked for compliance to pharmacopoeial standards. A list of excipients used, with their source and grade, is given in Table 3.3.

Table 3.3: Excipient selection

Sr.No.	Ingredients (Grade)	Pharmacopoeial Status	Manufacturer	Category
1	Xanthan Gum (FF, FFST)	USNF	Jungbauzlauer	pH-independent polymer
2	Sodium Alginate (Keltone HVCR) (Protanal LF 240 D)	USNF	ISP Alginates & FMC BioPolymer	pH-dependent polymer
3	Lactose Anhydrous (Pharmatose DCL 21)	USNF	DMV International	Diluent
4	Calcium Sulphate Dihydrate precipitated (Extra pure)	USNF	Merck	Matrix stabilizer
5	Hydroxypropyl Methylcellulose Phthalate (HP –55 S)	USNF	Shin-Etsu Chemi Co. Ltd	Enteric polymer
6	Hydroxypropyl Methylcellulose (K4M)	USNF	Dow	pH-independent polymer
7	Magnesium Stearate (Precipitated fine powder)	IP/USNF	Amishi Drugs & Merck	Lubricant
8	Opadry Blue (09 F-50878 Blue)	IH	Colorcon	Coating mix

3.4 Dosage design decision for OCRS-BID

The dosage design for OCRS-BID was based on the following 3 major performance characteristics:

i) *In-vitro* dissolution profile

ii) *In-vivo* pharmacokinetic study

iii) Accelerated and long term stability studies in line with ICH guidelines

Development of prototype formula for BID formulation was initiated using Xanthan gum or sodium alginate alone as the control release polymer using both dry granulation process and wet granulation process. In the wet granulation process, an aqueous dispersion of 1% Polyvinyl pyrrolidone K-30 (PVP K-30) was used as binder. The composition of the batches prepared by dry granulation process are shown in Table 3.4, the method of preparation thereunder and their dissolution profiles in Table 3.5.

Table 3.4: Composition of batches with single polymer using dry granulation process

Ingredient	**% w/w**	
Polymer	Xanthan gum	Sodium alginate
Cefaclor	74.17	74.17
Xanthan gum FFST	5.00	-
Sodium alginate (Keltone HVCR)	-	5.00
Anhydrous Lactose	18.82	18.82
Magnesium Stearate (intragranular)	1.00	1.00
Magnesium Stearate (extragranular)	1.00	1.00
Tablet weight	700mg	700mg

Cefaclor, polymer, Lactose and Magnesium Stearate were mixed, compacted, deslugged and lubricated using Magnesium Stearate. The lubricated granules were compressed into caplets of 18x8mm dimension.

Table 3.5: Dissolution profile of batch with single polymer using dry granulation process

Time	**% released**	
Polymer	Xanthan gum	Sodium alginate
1 hr	71.5	32.0
2 hr	93.5	55.0
3hr	-	91.0
4hr	-	94.0

The batch containing xanthan gum, showed more than desired release in both, the acidic and neutral conditions. Xanthan gum has a pH independent swelling profile. The dumping of the dose can be attributed to slow swelling of the polymer. The batch containing sodium alginate showed desired profile in acidic medium but faster profile (>90% in 3 hours)in the phosphate buffer. Sodium alginate has pH dependent solubility, with solubility increasing with increase in pH. Thus the matrix containing sodium alginate releases drug slowly in acidic pH and faster as the pH goes towards neutral range.

The composition of the batches prepared by wet granulation are shown in Table 3.6, the method of preparation thereunder and dissolution profiles in Table 3.7

Table 3.6 : Composition of batches with single polymer using wet granulation process

Ingredient	**%**	
Polymer	Xanthan gum	Sodium alginate
Cefaclor	74.17	74.17
Xanthan gum FFST	5.0	-
Sodium alginate (Keltone HVCR)	-	5.0
Anhydrous Lactose	18.82	18.82
PVP K30	1.0	1.0
Magnesium Stearate	1.0	1.0
Tablet weight	700mg	700mg

Cefaclor, polymer, Lactose were mixed, granulated with a dispersion of PVP in water, granules tray dried and lubricated using Magnesium Stearate. The lubricated granules were compressed into caplets.

Table 3.7 : Dissolution profile of batches prepared by wet granulation

Time	**% released**	
Polymer	Xanthan gum	Sodium alginate
1 hr	27.4	20.3
2 hr	39.9	33.2
3hr	56.6	41.3
4hr	73.1	58.0

Retardation in the release was more with wet granulation process than with dry granulation process. However the trend remained the same with the xanthan gum matrix showing a faster release rate than the sodium alginate matrix. Since cefaclor is reported[28] to undergo rapid loss of activity in neutral and alkaline solutions, wet granulation, using water as granulating fluid, would not be a preferred method for processing cefaclor formulations. Hence further batches were taken using the dry granulation process.

Keeping the formula as shown in Table 3.6 and using the dry granulation process, a batch was taken by reducing the concentration of Xanthan gum from 5% to 2%. The dissolution profile of this batch is shown in Table 3.8.

Table 3.8: Dissolution profile of batch with 2% Xanthan gum

Time	% released
1 hr	40.5
2 hr	48.0
3 hr	55.9
4 hr	56.8

By reducing the Xanthan gum concentration, the initial release was faster due to initial burst effect at acidic pH. However, after hydration of the gum, the drug release was retarded at a later stage.

From the above experiments it was evident that Xanthan gum was not capable of retarding release in acid but could retard release in neutral medium. Conversely, sodium alginate could retard release in acidic condition but owing to its solubility at increased pH, it caused dose dumping in phosphate buffer. This indicated that a single polymer could not tailor the profile to the desired one. However, if the two polymers were combined in a matrix, sodium alginate could retard the release in acidic medium and xanthan gum in the neutral medium and thus might give the desired dissolution profile.

Hence, a batch, containing a combination of Xanthan gum and Sodium alginate as per the the formula shown in Table 3.9 was prepared by the dry granulation process. The dissolution profile of this batch is shown in Table 3.10

Table 3.9: Batch containing sodium alginate and xanthan gum

Ingredients	**%w/w**
Cefaclor	76.35
Anhydrous Lactose	8.00
Microcrystalline cellulose (Avicel pH 101)	7.65
Xanthan gum FFST	2.00
Sodium Alginate (Keltone HVCR)	5.00
Magnesium Stearate	0.50
Magnesium Stearate	0.50
Tablet weight	680mg

Table 3.10: Dissolution profile of batch containing xanthan gum and sodium alginate

Time	% released
1 hr	48.1
2 hr	67.2
3 hr	83.5
4 hr	93.8

Although a desirable release profile was obtained after the 3rd hour, the initial burst effect could not be avoided. Xanthan gum of a higher viscosity (FF grade having viscosity of 1524 mPa.sec in place of FFST grade having viscosity of 1387mPa.sec) was used to overcome this initial burst. Accordingly, the concentration of Xanthan gum was reduced from 2% to 1%. Two levels of Sodium alginate (3% and 3.5%) were tried as shown in Table 3.11. The batches were prepared by the dry granulation process and the dissolution profiles are shown in Table 3.12

Table 3.11 : Batches containing sodium alginate and xanthan gum(FF)

Ingredient	%w/w	
Cefaclor	76.35	76.35
Xanthan gum FF	1.00	1.00
Sodium Alginate (Keltone HVCR)	**3.00**	**3.50**
Anhydrous Lactose	8.00	8.00
Avicel pH 101	10.60	10.10
Magnesium Stearate	0.50	0.50
Magnesium Stearate	0.50	0.50
Tablet weight	680mg	680mg

Table 3.12: Dissolution profile of batch containing xanthan gum(FF) and sodium alginate

Time	% released	
Sodium Alginate⇒	3%	3.5%
1 hr	52.5	40
2 hr	77.8	52
3 hr	91.8	75
4 hr	97.1	91

The batch containing 3% sodium alginate showed a faster dissolution profile than desired whereas that containing 3.5% showed a dissolution profile which almost matched the desired one. For assessing the stability of the formulation with respect to dissolution profile, the batch containing 3.5% sodium alginate was kept at 40°C/75% RH and at 60°C for 15 days. The dissolution profiles of the samples after 15 days at the storage conditions are shown in Table 3.13.

Table 3.13 Dissolution profiles of samples of BID formulation following stress testing

Time	% released		
	Initial	15 days at 60°C	15 days at 40°C/75% RH
1 hr	40	65	55
2 hr	52	92	75
3 hr	75	-	90
4 hr	91	-	-

With increasing temperature, the release also increased. This could be due to the reported[58] relaxation of sodium alginate on storage at high temperature. It was thus required to improve the gel structure of the matrix.

Sodium alginate is reported[61] to form a strong insoluble matrix gel in presence of divalent cations such as Barium and Calcium. Hence the addition of salts thereof in the formulation could improve the gel structure. Calcium Sulphate, Calcium Sodium Edetate, Calcium chloride, Calcium citrate and Calcium Stearate were evaluated, at a level of 14% of the sodium alginate concentration, as matrix stabilizers. The compositions of the resultant batches are shown in Table 3.14 and dissolution profiles of these batches are shown in Table 3.15

Table 3.14: Trials with different salts of Calcium

Ingredient	**%w/w**				
Cefaclor	74.87	74.87	74.87	74.87	74.87
Xanthan gum	1.00	1.00	1.00	1.00	1.00
Sodium alginate	3.50	3.50	3.50	3.50	3.50
Lactose	18.13	18.13	18.13	19.13	18.13
Calcium sulphate	**0.50**	-	-	-	-
Calcium chloride	-	**0.50**	-	-	-
Calcium Sodium EDTA	-	-	**0.50**	-	-
Calcium Stearate	-	-	-	**0.50**	-
Calcium citrate	-	-	-	-	**0.50**
Magnesium Stearate	1.00	1.00	1.00	1.00	1.00
~~Magnesium~~ Stearate	~~1.00~~	1.00	1.00	1.00	~~1.00~~
Tablet weight	700mg	700mg	700mg	700mg	700mg

Table 3.15: Dissolution profiles of batches containing different salts of Calcium

Time	% released				
	$CaSO_4$	**$CaCl_2$**	**Ca.Na Edetate**	**Ca Stearate**	**Ca Citrate**
1 hr	30.9	Tablets disintegrated immediately	31.1	43.5	37.7
2 hr	47.1		47.7	53.6	52.4
3 hr	68.8		69.7	69.1	71.5
4 hr	83.4		88.8	84.6	87.1

All the calcium salts used, other than Calcium chloride, seemed to give a dissolution profile similar to the desired one. Tablets containing calcium chloride disintegrated immediately on contacting the dissolution medium.

To observe the effect of Calcium salts on stabilization of dissolution profile on storage, all the batches, except the one containing calcium chloride, were kept at stress condition of 60°C. The dissolution profiles before and after 7 days at the stress condition are shown in Table 3.16.

Table 3.16: Dissolution profiles of batches containing different salts of calcium after stress studies

Time	% released							
	CaSO4		**Ca.Na Edetate**		**Ca Stearate**		**Ca Citrate**	
	Initial	**7 days**	**Initial**	**7 days**	**Initial**	**7 days**	**Initial**	**7 days**
1 hr	30.9	43.7	31.1	63.2	43.5	78.5	37.7	76.9
2 hr	47.1	53.1	47.7	82.7	53.6	95.9	52.4	95.7
3 hr	68.8	71.1	69.7	97.8	69.1		71.5	
4 hr	83.4	86.3	88.9		84.6		87.5	

Among all the calcium salts used, only calcium sulphate was able to maintain the desired dissolution profile even after 7 days at the stress condition.

In order to optimize the concentration of calcium sulphate, batches containing 7, 14, 28 and 98 % of Calcium sulphate of the weight of sodium alginate added (3.5%) were prepared. The batches were kept at the stress condition of 60°C for 7 days. The dissolution profile before and after 7 days at the stress condition are shown in Table 3.17.

Table 3.17: Dissolution profiles of batches containing varying concentrations of calcium sulphate after stress studies

Time⇓ CaSO4⇒ (% of sodium alginate)	% released							
	7%		14%		28%		98%	
	Initial	7 days	Initial	7 days	Initial	7 days	Initial	7 days
1 hr	33.0	52.5	30.9	43.7	35.0	60.9	40.2	79.5
2 hr	54.0	77.1	47.1	53.1	46.4	69.0	44.8	82.3
3 hr	75.4	93.4	68.8	71.1	63.0	83.0	48.9	88.0
4 hr	95.3	-	83.4	86.3	78.7	96.0	53.2	90.5

Out of the four levels of calcium sulphate evaluated, only 14% of calcium sulphate was able to maintain the dissolution profile after 7 days at 60°C. However, the release in the first hour did not comply to the desired specifications inspite of addition of calcium sulphate. A batch was taken by increasing the level of xanthan gum from 1 % to 1.5% and that of sodium alginate from 3.5% to 4% maintaining the level of calcium sulphate at 14% of the weight of sodium alginate. The dissolution profile of this batch before and after stress studies is shown in Table 3.18

Table 3.18: Dissolution profile of batch containing increased level of polymers with calcium sulphate after stress studies

Time	% released	
	Initial	7 days
1 hr	28.6	39.6
2 hr	45.6	50.8
3 hr	69.8	70.6
4 hr	80.3	84.5

Increasing the level of polymers in the matrix could not tailor the release to the desirable specification in the first hour, in acidic medium, after exposure to the stress condition. This called for addition of an enteric polymer in the tablet matrix. Hydroxypropyl Methylcellulose phthalate(HPMCP) was tried at the level of 2, 4 and 6%. The batches were granulated with a solution of HPMCP in Isopropyl alcohol: methylene chloride mixture (1:3). The dissolution profile of these batches are shown in Table 3.19

Table 3.19: Dissolution profiles of batches granulated with Hydroxypropyl Methylcellulose phthalate

Time⇓ HPMCP⇒	**% released**	
	2%	4%
1 hr	45.3	26.2
2 hr	66.1	38.4
3 hr	81.0	55.8
4 hr	90.0	72.7

2% HPMCP was not able to retard the release to the desired extent while 4 % brought about considerable retardation of release. Since enteric polymers are usually cured, a portion of the batch prepared using 4% HPMCP was heated at 60°C for 24 hours while the other portion was kept as such. The cured and non-cured tablets were subjected to stress testing at 60°C for 7 days. The release profile before and after stress testing is shown in Table 3.20.

Table 3.20: Effect of curing on dissolution profile

Time	% released			
	Without curing		With curing	
	Initial	7days	Initial	7days
1 hr	26.2	35.0	34.0	36.2
2 hr	38.4	57.0	55.0	60.5
3 hr	55.8	73.2	74.2	75.0
4 hr	72.7	92.6	92.0	93.7

The release profile was unchanged after stress testing in the tablets which were cured. Hence it was decided to cure tablets after coating them.

Table 3.21 shows the optimized formula for BID tablets and the method of preparation thereunder.

Table 3.21: Final prototype unit formula for OCRS-BID

Ingredient	Mg/tablet
Cefaclor Equivalent to anhydrous cefaclor	527.17 500mg
Xanthan gum FF	10.5
Sodium Alginate (Keltone HVCR)	28.0
Anhydrous Lactose	95.97
Calcium sulphate	3.36
Hydroxypropyl Methylcellulose phthalate	28.0
Magnesium Stearate	7.0
Methylene chloride	0.15mL
Isopropyl alcohol	0.05mL
Opadry 09F50878 Blue	26.25
Purified water	qs

Procedure

a) Sift Cefaclor, Xanthan gum, sodium alginate, anhydrous lactose and calcium sulphate dihydrate through #40 screen.

b) Blend the sifted material of Step a in a rapid mixer granulator.

c) Disperse HPMCP in the mixture of isopropyl alcohol and methylene chloride and add to blend in rapid mixer granulator to get granules.

d) Dry the granules in fluidized bed dryer at 45-50°C till the loss on drying is between 2-3.5%.

e) Pass the dried granules of step d through turbo sifter fitted with 1mm sieve.

f) Blend the sized granules in octagonal blender with Magnesium Stearate previously sifted through 40# screen.

g) Compress the lubricated granules into caplets.

h) After satisfactory results on core tablets, film coat the tablets with aqueous dispersion of Opadry until 3% weight gain is obtained.

i) Load the coated tablets in a tray dryer, set at 60°C, and cure the tablets for 24 hours.

j) After satisfactory results of coated tablets, pack them.

3.4.1 Acclerated stability studies

3 batches were prepared using the formula as shown in Table 3.21 and kept at accelerated (40°C/75%RH) and long term (25°C/60%RH) storage conditions for 3 months, in-line with ICH guidelines, after packing in aluminum strips. The results of these studies are appended as Annexure 3.

As can be seen from the results of the accelerated stability study, although the release of Cefaclor becomes faster after one hour, with time at the accelerated condition, it is within the acceptance limit of 15-55%. Complete release (>90%) is observed at the end of 4 hours in both the storage conditions at the end of 3 months of study. The drop in the assay was found to be within the acceptance limit of 90-115% after 3 months at both the conditions. There is no significant water uptake which indicates that the pack is adequately protecting the product against moisture pick-up.

3.4.2 Bioequivalence studies

A batch was prepared using the formula as shown in Table 3.21 and studied (open label, balanced, randomized, two treatment, two-period, two sequence, single-dose, crossover) for bioequivalence against Elli Lilly's(USA) Ceclor CD 500mg, in fed and fasted condition, in 8 healthy human volunteers at Lambda Therapeutic Research Pvt. Ltd., Ahmedabad, India.

In the fasted study, all the subjects were required to fast overnight, atleast 10-12 hours before the scheduled time for the dose administration and for four hours post-dose. Drinking water was not allowed from one hour before dosing until two hours post-dose. In the fed study, after an overnight fast of at least 10-12 hours, a standard breakfast was provided to each volunteer.

The dose was administered within 5 minutes of completion of the breakfast. Drinking water was not allowed till two-hours post-dose. Prior to and thereafter, it was allowed at all times. In both, the fed and fasted studies, standard meals were provided to the subjects 4 hours after dosing and at specified intervals from then on till checkout (10 hours). The test and reference formulations were administered to the subjects while in sitting posture alongwith 240mL of drinking water at ambient temperature and they were instructed to remain seated or ambulatory for the first three hours following the administration of each drug. Thereafter they were allowed to engage in normal activities. A wash out period of atleast 3 days was kept between the administration of test and reference products.

A total of 6mL blood samples were collected during each period. The venous blood samples were withdrawn pre-dose and at 0.5, 0.75, 1, 1.25, 1.5, 1.75.2.0,2.5,3.0,4.0,6.0,8.0 and 10 hours following drug administration. Samples were collected through an in-dwelling cannula placed in a forearm vein. The plasma from the samples collected was assayed for cefaclor using a validated HPLC method. T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, λz and $t_{1/2}$ for cefaclor were calculated using WinNonlin Professional software (Scientific Consulting Inc., USA). The untransformed and log-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were subjected to analysis of variance, Two one-sided tests for bioequivalence, 90% confidence intervals for the difference of means between drug formulations were calculated for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ using both untransformed and log transformed data. The products were to be considered bio-equivalent if the 90% confidence intervals of log-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were between 80-125%.

The results of the fasted and fed study are shown in Annexure 4 and 5 respectively.

4

EXPERIMENTAL-II

CEFACLOR OCRS-OD WITH OR WITHOUT PROBENECID

4.1 Dosage design decision for OCRS-OD

The dosage design for OCRS-OD was based on the following 3 major performance characteristics:

iii) *In-vitro* dissolution profile

iv) *In-vivo* pharmacokinetic study

iii) Accelerated and long term stability studies in line with ICH guidelines

OD products with strength 1500mg (750mgx2) and 1000mg equivalent to 500mg TID and Ceclor CD 500 mg respectively were formulated.

Based on the dissolution profile of the BID product, it was decided to prepare a product with a 12 hour dissolution profile such that 20-40% cefaclor would get released in 2 hours, 45-65% in 6 hours and NLT 90% in 12 hours. The materials used in the development of this product are same as those used in the development of BID tablets.

4.1.1 Drug products containing xanthan gum and sodium alginate

Tablets containing 750mg of Cefaclor were prepared by dry granulation process, using Xanthan gum and Sodium alginate as the release retarding polymers. The formula for this batch is shown in Table 4.1. The dissolution profile of this batch is shown in Table 4.2.

Table 4.1: Composition of OD tablets (750mg) containing xanthan gum and sodium alginate (Batch No.: CSK(46)127)

Ingredient	**%w/w**
Cefaclor	74.56
Sodium alginate	5.00
Xanthan gum	5.00
Lactose anhydrous	13.44
Magnesium Stearate	1.00
~~Magnesium Stearate~~	~~1.00~~
Tablet weight	1050mg

Table 4.2: Dissolution profile of OD tablets (750mg) containing xanthan gum and sodium alginate

Time (hrs)	**% release**
1	12.3
2	21.3
3	27.0
4	33.8
5	42.0
6	50.7
7	59.0
8	66.9
9	75.1
10	83.6
11	89.9
12	93.8

On careful observation of the behavior of the tablets in the dissolution vessel, they were found to form a very soft irregular mass on contacting the dissolution medium. The release was spread evenly across 12 hours and complied to the targeted profile.

The batch was packed in PVdC (90) gsm coated PVC blisters and HDPE bottles and kept for stability studies, at accelerated (40°C/75%RH) and long term (25°C/60%RH) conditions. The stability data is appended as Annexure 6. However, within one month, complete release of the drug took place within 6 hours instead of the targeted 12 hours in both the packs studied. This could be due to instability in gel structure at elevated temperature. As observed in the BID tablets, stabilization of gel structure was desirable to extend the release. Hence as per the experience in BID tablet development, it was decided to add Calcium sulphate to further batches of the OD tablets.

The tablets picked up about 1% moisture in one month indicating that the packs were not protective enough. Hence further batches of product were to be packed in more moisture resistant packs.

Pharmacokinetic studies of this batch conducted at Lambda Therapeutic Research Pvt. Ltd., Ahmedabad, India. Two tablets of 750mg each, were administered to 8, fed, healthy human volunteers . The results of the study are appended as Annexure 7. For comparison, the bioavailability of conventional capsules of cefaclor (Distaclor 500mg of Ranbaxy Labs. Ltd.) was also studied in 8, fed, healthy human volunteers. The results of this study are appended as Annexure 8.

Extrapolating the pharmacokinetic parameters obtained on administering 1 capsule of Distaclor (500mg) to 3 capsules, the T/R values for the pharmacokinetic parameters for 2 OD tablets of 750mg were calculated and are shown in Table 4.3

Table 4.3: T/R for 2x750mg OD tablets Vs 3x500mg conventional capsules

Parameter	T/R
Cmax	91.22
AUC_{0-t}	67.70
$AUC_{0-\infty}$	64.86

Although the T/R for Cmax is within 80-120%, that for AUC $_{0-t}$ and $AUC_{0-\infty}$ is below this limit. This indicates that the rate of release from OD tablets is close to desirable but bioavailability was lower as compared to conventional capsules. The T>MIC(1μg/mL), calculated using the formula as shown in Section 2.1.3.1.2, for this batch was 52.76% as compared to IR formulation, which complies to the requirement of >40% to ensure a bacteriologic cure of over 90%. The $t_{1/2}$ of the OD product was found to be 1.960 hours as compared to 0.774 hours of the conventional capsules indicating that absorption takes place in the OD product even after Tmax (6.75hrs) is reached. However, the product seems to cross the absorption window before releasing the entire drug load and hence there is decreased bioavailability.

In order to ensure that entire drug is released within the absorption window region, it was decided to prepare a formula having 6 hour dissolution profile and also investigate the effect of reducing dose of the OD product on the *in-vivo* behavior.

4.1.2 Drug products containing Hydroxypropyl Methylcellulose and calcium sulphate

To rectify the problem of faster dissolution rate on storage and low bioavailability, calcium sulphate and Hydroxypropyl Methylcellulose (K4M/K15M) were added in the formula. All the batches shown in Table 4.4 contained calcium sulphate equivalent to 14% of the quantity of sodium alginate added. The dissolution profile of these batches is shown in Table 4.5.

Table 4.4 : Composition of OD tablets (750mg) containing HPMC and calcium sulphate

Ingredients⇓	**%w/w**		
Batch No.⇒	**SPB(72)147A**	**SPB(72)151**	**SPB(72)157A**
Cefaclor	75.20	75.20	75.20
Sodium alginate	5.00	3.50	4.00
Xanthan gum	2.00	1.00	1.50
Hydroxypropyl Methylcellulose K4M	3.00	2.00	2.00
Calcium sulphate	0.70	0.49	0.56
Lactose anhydrous	12.09	15.84	15.13
Magnesium Stearate	1.00	1.00	1.00
Magnesium Stearate	1.00	1.00	1.00
Tablet weight	1050mg	1050mg	1050mg

Table 4.5 : Dissolution profile of OD tablets(750mg) containing HPMC and calcium sulphate

Time (hrs)	% release		
	SPB/72/147	SPB/72/151	SPB/72/157A
1	16.8	24.0	22.4
2	22.4	37.5	35.7
3	33.6	55.9	57.2
4	45.5	71.0	73.7
5	-	83.4	85.7
6	-	91.6	91.9

Keeping the formula same as that of batch CSK/46/127, except for the addition of calcium sulphate and replacing 60% of the quantity of xanthan gum with HPMC K4M, the release was found to be much retarded with only 45.5% getting released after 4 hours (Batch SPB/72/147). To accelerate the release rate, quantities of Sodium alginate, HPMC, xanthan gum were reduced in batches SPB/72/151 and SPB/72/157A. A satisfactory dissolution profile was obtained. The tablets of batch SPB/72/157A maintained their integrity for longer period than those of batch SPB/72/151.

Batches were also prepared for 1000mg strength of cefaclor tablets. The formula of these batches is shown in Table 4.6 and their dissolution profile in Table 4.7.

Table 4.6: Composition of tablets of 1000mg strength

Ingredient⇓ Batch⇒	%w/w		
	SPB(72)161B	SPB(72)167B	SPB(72)175
Cefaclor	74.79	72.18	74.79
Sodium Alginate	4.00	3.35	2.40
Xanthan Gum	1.50	1.44	1.00
Hydroxypropyl Methylcellulose K4M	2.00	2.00	2.00
Calcium Sulphate	0.56	4.00	0.30
Lactose anhydrous	15.15	15.15	17.14
Magnesium Stearate	1.00	0.95	1.00
Magnesium Stearate	1.00	0.95	1.00
Weight of tablet	1400mg	1458.63mg	1400mg

Table 4.7: Dissolution profile of batches of 1000mg strength

Time (hrs)	% release		
	SPB(72)161B	SPB(72)167B	SPB(72)175
1	16.5	20.2	22.4
2	26.8	33.0	35.7
3	41.9	48.7	57.1
4	55.7	60.9	64.2
5	67.2	70.8	77.4
6	76.5	81.3	86.3
7	-	89.2	-

Formula for batch SPB/72/161B was exactly in line with that of batch SPB/72/157A. However its dissolution profile was slower and complete release was not obtained by 6 hours. Batches SPB/72/167B and SPB/72/175 contained lower concentrations of the polymers and gave a desirable rate and extent of release. The tablets of both the batches maintained their integrity in the dissolution medium. Since batch SPB/72/175 contained lower concentration of polymers as compared to batch SPB/72/167B, it was preferred for further studies.

Batches SPB/72/157A and SPB/72/175 were packed in Aclar-PVC blisters and kept for stability studies, at accelerated (40°C/75%RH) and long term (25°C/60%RH) conditions. The stability data is appended as Annexure 9. In batch SPB/72/157A (750mg strength), the release went on increasing at every month at the accelerated storage condition with complete release taking place within 4 hours at the three month time point. Moisture pick-up was not very remarkable after 3 months indicating that the pack used was protective enough. In batch SPB/72/175(1000mg strength), the entire dose got released at the 5 hour time point after one month at the accelerated storage condition. As in the case of the BID tablets, since the release profile was reduced to only 6 hours, the formulation would stay in the acidic pH for a longer duration and hence required an enteric polymer to retard release. Hence it was decided to use Hydroxypropyl methylcellulose phthalate in future batches.

Pharmacokinetic studies for tablets of batches SPB/72/157A and SPB/72/175 were conducted at Lambda Therapeutic Research Pvt. Ltd., Ahmedabad, India. The study (open label, balanced, single period, two treatment, single dose) was carried out with two tablets of 750mg or one of 1000mg administered per volunteer. 8, fed, healthy human volunteers were assigned for each strength. The results of the study are appended as Annexure 10.

Extrapolating the pharmacokinetic parameters obtained on administering 1 capsule of Distaclor (500mg) to 3 capsules, the T/R values for the pharmacokinetic parameters for 2 tablets of 750mg were calculated and are shown in Table 4.8

Table 4.8: T/R for 2x750mg OD tablets against 3x500mg conventional capsules

Parameter	T/R
Cmax	171.93
AUC $_{0-t}$	86.30
$AUC_{0-\infty}$	85.38

Although the T/R for AUC $_{0-t}$ and $AUC_{0-\infty}$ were within 80-120%, that for Cmax was well above this limit. This indicates that the OD tablets have comparable bioavailability as compared to 3 conventional capsules but that dose dumping takes place. The $t_{1/2}$ for this batch was very similar to that of the conventional capsule (0.869 Vs 0.774h) indicating that little drug got absorbed after T_{max} (5.75h) was reached. The T>MIC(1μg/mL) value was 35.10% which is below the required value of >40% to ensure 90% bacteriological cure. This indicated that the drug got absorbed in too short a period of time and hence got eliminated quickly thus reducing T>MIC. This indicated that a release profile of 6 hours was too short and that for 12 hours was long. Hence it was decided to prepare tablets with release profile of 10 hours.

Extrapolating the pharmacokinetic parameters obtained on administering 1 capsule of Distaclor (500mg) to 3 capsules, the T/R values for the pharmacokinetic parameters for one tablet of 1000mg were calculated and are shown in Table 4.9

Table 4.9: T/R for 1x1000mg OD tablet against 3x500mg conventional capsules

Parameter	T/R
Cmax	135.95
AUC_{0-t}	65.65
$AUC_{0-\infty}$	66.29

Although the Cmax was not as high as that observed on administering 2 x750mg tablets, it was not within the acceptable 80-120% range. This indicated that the drug was getting released within the absorption window region. However, AUC_{0-t} and $AUC_{0-\infty}$ were much below the desired 80-120% range indicating lower bioavailability although the $t_{1/2}$ of this product was higher than that of conventional capsule (1.277 Vs 0.774h) indicating absorption was taking place even after T_{max} was reached. The T>MIC(1µg/mL) value was 30.60% which is below the required value of >40% to ensure 90% bacteriological cure. These parameters indicated that a dose of 1000mg may not be enough to make it a OD formulation. For making this dose work, it was required to prolong the release profile and reduce the elimination of the drug by co-administering a drug like probenecid.

4.1.3 Drug products containing Hydoxypropyl Methylcellulose phthalate

Although addition of Hydroxypropyl Methylcellulose retarded the release *in vitro*, it was not effective in doing so *in vivo*. Hence it was replaced by enteric polymer Hydroxypropyl Methylcellulose phthalate. 4% of this polymer was used in the BID formulation. Due to increased dose of the drug in the OD formulation, the proportion of HPMCP was increased to 6% in the OD tablets. For prolonging the drug release upto 10 hours, the quantity of sodium alginate and xanthan gum were increased. The composition of various batches is shown in Table 4.10 and their dissolution profile in Table 4.11.

Table 4.10 : Composition of OD tablets (750mg) containing HPMCP

Ingredient⇓ Batch⇒	%w/w		
	SPB(99)37	SPB(99)39	SPB(99)45
Cefaclor	75.42	75.42	75.42
Sodium Alginate	6.00	8.00	8.00
Xanthan Gum	3.00	3.00	4.00
Hydroxypropyl Methyl-cellulose Phthalate	6.00	6.00	6.00
Calcium Sulphate	0.72	0.96	0.96
Lactose anhydrous	7.85	5.61	4.61
Magnesium Stearate	1.00	1.00	1.00
Weight of tablet	1050mg	1050mg	1050mg

Table 4.11 Dissolution profile of batches containing HPMCP

Time (hrs)	% release		
	SPB(99)37	SPB(99)39	SPB(99)45
1	17.6	15.1	15.6
2	23.9	20.7	19.2
3	36.7	31.9	28.7
4	51.2	45.5	40.3
5	67.5	60.1	52.0
6	81.1	72.3	64.6
7	91.0	81.8	74.4
8	96.9	88.8	82.7
9	-	96.1	87.2
10	-	-	89.2

The release profile of the batch SPB/99/45 was satisfactory.

Curing of tablets was found to maintain the dissolution profile of BID tablets even after 3 months at the accelerated condition. Hence the tablets of batch SPB/99/45 were cured at 50°C for 24 hours. The dissolution profile of the batch after curing is shown in Table 4.12.

Table 4.12 Dissolution profile of batch SPB/99/45 before and after curing

Time (hrs)	% release	
	Before curing	After curing
1	15.6	16.2
2	19.2	23.4
3	28.7	35.7
4	40.3	48.4
5	52.0	61.5
6	64.6	74.7
7	74.4	85.7
8	82.7	92.1
9	87.2	-
10	89.2	-

Curing was found to increase the release rate and curtail the dissolution profile to 8 hours. Batches as per composition shown in Table 4.13 were taken and their dissolution profile studied after curing for 24 hours at 50°C. The dissolution profiles are shown in Table 4.14.

Table 4.13 Compostion containing HPMCP

Ingredient⇓ Batch⇒	%w/w	
	SPB(99)55B	SPB(99)63
Cefaclor	75.23	75.23
Sodium Alginate	6.00	5.00
Xanthan Gum	6.00	5.00
Hydroxypropyl Methylcellulose Phthalate	6.00	6.00
Calcium Sulphate	0.72	0.60
Lactose anhydrous	5.05	7.17
Magnesium Stearate	1.00	1.00
Tablet weight	1050mg	1050mg

Table 4.14 Dissolution profile of batches containing HPMCP after curing

Time (hrs)	% release	
	SPB(99)55B	SPB(99)63
1	16.0	17.1
2	19.6	21.4
3	25.1	30.4
4	33.6	39.0
5	41.7	47.4
6	52.2	56.0
7	63.3	64.6
8	77.3	72.8
9	85.6	80.3
10	91.2	87.5

Tablets of batch SPB/99/63 were packed in Aluminum strips and kept for stability at both accelerated (40°C/75% RH) and long term (25°C/60% RH) conditions for 3 months. The stability data is attached as Annexure 11. The release profile remained in the desired range even after 3 months at the accelerated storage condition in Aluminum strips. The assay was within the acceptance criteria of 90-110% after 3 months at both, the accelerated and long term condition. No significant change occurred in the water content of the product during this study indicating that the pack was protective enough.

Tablets of batch SPB/99/63 were studied for bioavailability at Lambda Therapeutic Research Pvt. Ltd., Ahmedabad, India. The study (open label, randomized, balanced, three-treatment, three sequence, single dose, crossover)was carried out with two tablets of 750mg administered to each of the 12 healthy, fed human volunteers. The results of the study are appended as Annexure 12.

Extrapolating the pharmacokinetic parameters obtained on administering 1 capsule of Distaclor (500mg) to 3 capsules, the T/R values for the pharmacokinetic parameters for 2 tablets of 750mg were calculated and are shown in Table 4.15

Table 4.15: T/R for 2x750mg OD tablets against 500mg single dose x 3

Parameter	T/R
Cmax	102.41
AUC0-∞	83.50
ln transformed least squares means	
$AUC_{0-\infty}$	84.9
Partial AUC	81.6

The T/R for C_{max}, and $AUC_{0-\infty}$ were within 80-120%. This indicates that the OD tablets have comparable bioavailability as compared to conventional capsules administered three times a day. The T>MIC(1μg/mL) value was 55.125% as compared to the conventional product, which satisfies the requirement of >40% to ensure 90% bacteriological cure.

4.1.3.1Pharmacokinetic study of OCRS-OD with Probenecid

A batch on the lines of the formula of batch SPB/99/63 was taken for 500mg strength. The bioavailability of Cefaclor from 2 tablets of 500mg and 2 tablets of conventional Probenecid tablets was studied in 12 fed, healthy human volunteers. The results of the study are appended as Annexure 13. Extrapolating the pharmacokinetic parameters obtained on administering 1 capsule of Distaclor (500mg) to 3 capsules, the T/R values for the pharmacokinetic parameters for 2x500mg OD tablets + 2x500mg Probenecid tablets were calculated and are shown in Table 4.16

Table 4.16: T/R for 2x500mg OD + 2x500mg Probenecid tablets against 500mg single dose x 3

Parameter	T/R
C_{max}	128.68
AUC $_{0-t}$	104.84
$AUC_{0-\infty}$	116.70

Although AUC $_{0-t}$ and $AUC_{0-\infty}$ were within 80-125%, the C_{max} was beyond this range even when the dose of Cefaclor was lower than that in the pervious study (1g vs 1.5g). This could be attributed to the prevention of excretion of cefaclor by probenecid. T>MIC(1μg/mL) value was 57.375% as compared to conventional product, which complies to the requirement of >40% to ensure 90% bacteriological cure.

5

DISCUSSION

The conventional dose of cefaclor is 1 to 2 gm daily, given in divided doses at 8 or 12 hourly intervals. It shows high peak level immediately after administration due to the rapid absorption of the drug followed by rapid fall of the blood level below the MIC due to high elimination rate, leading to very short period of exposure of the drug above its MIC level and thus requiring frequent administration of the dose to maintain the blood level above MIC.

The objective of the present study was to administer the drug in a manner that it achieves twice a day and once a day profile either through *in-vitro* intervention i.e. by extending the release profile of the drug substance from the delivery system, wherein the amount of the active incorporated in the hydrophilic polymer matrix is higher than that in the conventional products available; OR through combination of *in-vitro* and *in-vivo* interventions, wherein owing to probenecid's ability to reduce the tubular excretion of the drug substance, the amount of drug substance incorporated in the hydrophilic polymer matrix can be reduced as compared to that required without probenecid.

Since antibiotics are high dosing and high frequency drugs, extended release drug delivery systems thereof, need high doses of drug substance to be incorporated in a unit dosage form. Prior art suggests the use of high levels of polymers to achieve desirable rate controlled release profiles. This increases the volume of a dosage unit to unacceptable size and hence there have been relatively few successes in developing controlled release products for high dose/high frequency drugs. In the present study, a combination of biodegradable xanthan gum and sodium alginate, at fairly low levels, were used as extending polymers to achieve desirable release profiles.

The pH as well as other physical and chemical variables in solution have different effects on the gel properties of xanthan gum and sodium alginate, which are termed major factors in controlling the drug release mechanism. Xanthan gum is compatible with virtually all salts and solution pH and temperature have very little effects on the viscosity of its gel. It swells considerably[72] even at very low concentrations hence including it in the formulation reduces the load of polymer in the matrix and hence helps in keeping the tablet size smaller. It has been reported [73] that when tablets containing only xanthan gum as the extending polymer are surrounded by an aqueous environment, the surface of individual tablets tends to hydrate and form a gel layer to prevent rapid penetration of solution into the inner layer. After drug leaches out of the gel layer, an obvious sharp boundary forms to separate the dry core from the swollen and drug depleted gel layer. The size of the tablet increases significantly due to the polymer hydration and remains one complete gel unit after drug releases out. In contrast to xanthan gum, the gel rheological properties of alginate are highly dependent on pH and types of ion present in solution. The gel swelling process is not observed with alginate tablets in acidic media. Due to chemical conversion of sodium alginate to insoluble and nonswellable alginic acid by acidity, tablets appear unswollen and porous in acid environment. However, the tablets swell as the pH increases. Calcium salts cause cross linking in alginate gels as a result of which, the gels resemble a solid in retaining their shape and resisting stress.

Hydrophilic gums are reported to form swellable matrix. The nature of drug release was characterized by fitting the drug release data into various mathematical models.

5.1 *In-vitro* data analysis for OD matrix

OD tablets of 3 distinct dissolution profiles, as shown in Table 5.1, varying in their duration, i.e. 6 hours, 10 hours and 12 hours, were optimized during this study. The dissolution profiles were determined by using 0.1NHCl as the dissolution medium for the first two hours and then replacing it with pH 6.8 phosphate buffer for the remaining time interval.

Table 5.1: Release profiles of OD formulations developed

Time in hours↓ OD product with→	**Amount released (mg)**		
	6 hr profile	**10hour profile**	**12 hour profile**
1	168.00	128.25	92.25
2	267.75	160.50	159.75
3	429.00	228.00	202.50
4	552.75	292.50	253.50
5	642.75	355.50	315.00
6	689.25	420.00	380.25
7		484.50	442.50
8		546.00	501.75
9		602.25	563.25
10		656.25	627.00
11			674.25
12			703.50

5.1.1 Order of release

To determine whether the release from the tablets is zero-order or first order, the amount of drug released was plotted against time and log of amount of drug released was plotted against time. The plots are shown as Figs. 5.1 and 5.2 respectively.

Fig 5.1: Zero order release plot for OD formulations

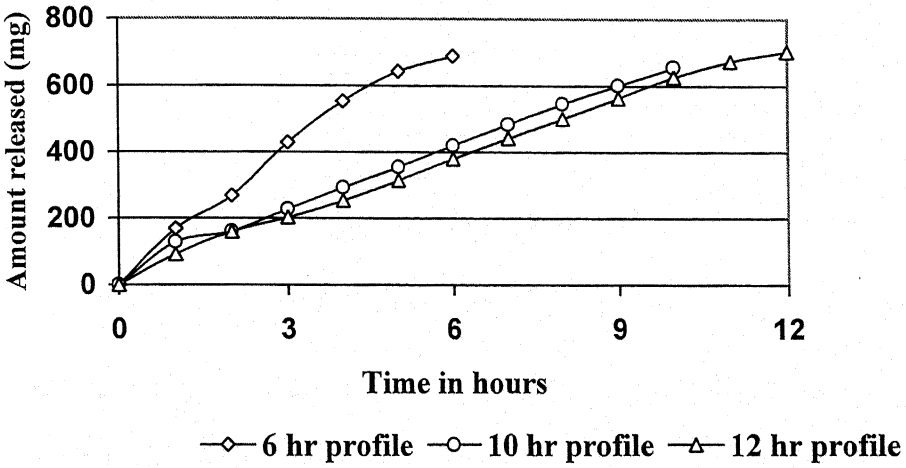
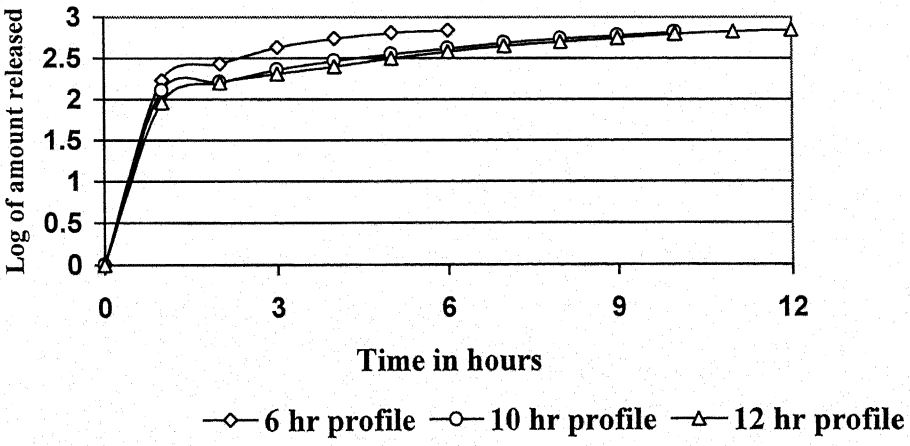


Fig. 5.2: First-order release plot for OD formulations



A linear relation was seen in Fig. 5.1 but not in Fig. 5.2. The correlation coefficient (r^2) values for the curves in Fig. 5.1 are shown in Table 5.2.

Table 5.2: Correlation coefficient values for release profile of OD Formulations

OD Formulation	r^2
6 hr profile	0.971
10 hr profile	0.9859
12 hr profile	0.993

The fact that the r^2 values tended to 0.99 indicated that the release from all the formulations follows zero order kinetics i.e. the release is independent of the pH of the environment and the amount of drug in the matrix.

5.1.2 Mechanism of release

To determine the nature of drug release, the amount of drug released was plotted against square root of time as shown in Fig. 5.3. The correlation coefficient values for the curves obtained are shown in Table 5.3.

Fig 5.3: Amount of drug released Vs $t^{1/2}$ plot for OD formulations

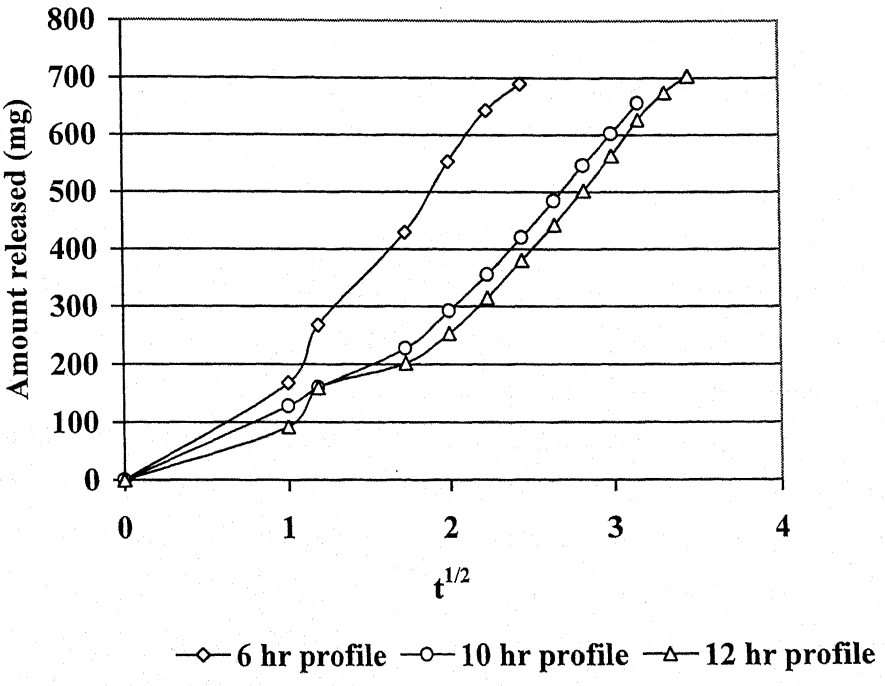


Table 5.3: Correlation coefficient values for diffusion controlled matrix model

OD Formulation	r^2
6 hr profile	0.9574
10 hr profile	0.9265
12 hr profile	0.9015

The fact that the r^2 values for zero order release tend more towards 0.99 than those seen in Table 5.2 indicates that the release cannot be fitted into Higuchi's model for diffusion controlled systems[74]. Rather the release is zero-order.

In the equation

$$\frac{M_t}{M_\infty} = Kt^n$$

if n = 0.5 then release is Fickian, if n= 1 release is case II and if it is between 0.5 and 1 then the release is non-Fickian[75]. The plots for n=1 and n=0.5 are already shown in Figs. 5.1 and 5.3. Since the plots of drug released Vs $t_{1/2}$ are not straight lines, it is confirmed that the release is not Fickian. It could either be by case II transport or show anomalous or non-Fickian behavior. It is reported that Anomalous behavior is exhibited by zero order release systems where the prevailing molecular mechanism is a coupling of diffusion and macromolecular relaxation as a result of which the drug diffuses outward with a kinetic behavior that is independent on the relative ratio of diffusion and relaxation.

5.2 Stability of drug product

The matrix (BID/OD)containing only sodium alginate and xanthan gum was unable to maintain the dissolution profile when exposed to elevated conditions of temperature and humidity for 15 days. This could be attributed to relaxation of sodium alginate at higher temperature. To improve the gel structure, various calcium salts were tried and calcium sulphate, at a level of 14% of the sodium alginate added, was found to afford maximum protection to the dissolution profile following stress testing. However the profile was faster than desirable in the first hour and hence various levels of Hydroxypropyl Methylcellulose phthalate were tried. HPMCP, 4% for BID and 6% for OD, was found to give a desirable dissolution profile even after curing the product at 60°C for 24 hours. The optimized formula, when studied for stability at accelerated (40°C/75%RH)and long term(25°C/60%RH) conditions as recommended in ICH guidelines, complied to the requirements of assay, dissolution profile and water content after 3 months in Aluminum strip pack. This would predict a room temperature shelf life of approximately 24 months.

5.3 *In-vivo* data analysis

5.3.1 BID matrix

A cross-over bioequivalence study was conducted for the optimized BID formulation against reference product, Ceclor CD (Eli Lilly, USA) in fed and fasted conditions. The *in-vivo* profiles obtained in this study are shown in Figs.5.4 and 5.5.

Fig 5.4: *In-vivo* plasma profile of Ceclor CD and Cefaclor BID in fasted volunteers

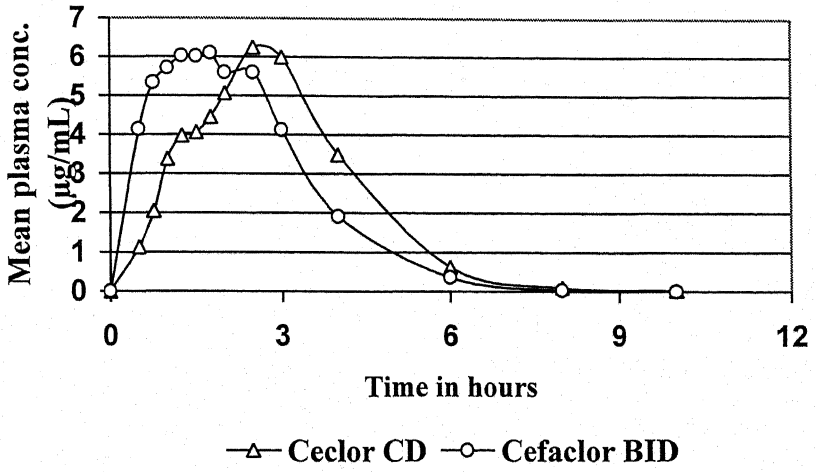
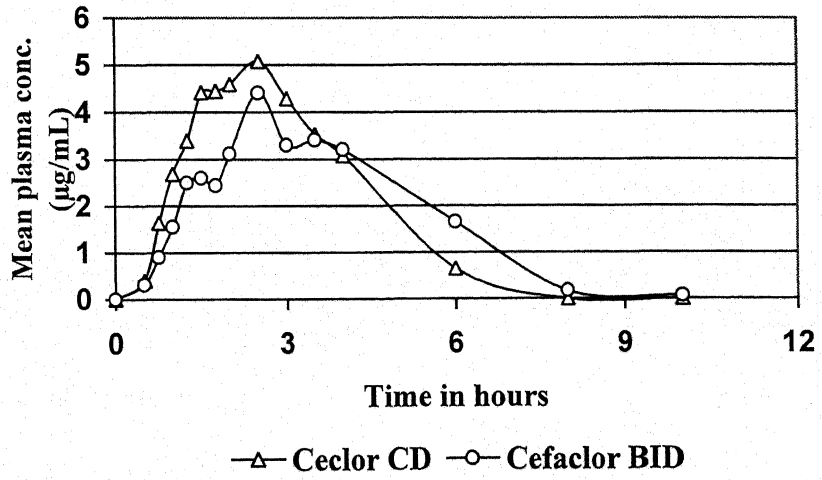


Fig 5.5: *In-vivo* plasma profile of Ceclor CD and Cefaclor BID in fed volunteers



The summary of the pharmacokinetic parameters obtained in the cross over study in fed and fasted volunteers are shown in Table 5.4 and Table 5.5.

Table 5.4 :Summary of pharmacokinetic parameters of BID product in fasted study

Measures		T_{max}	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$
Test Formulation (B)					
N		8	8	8	8
Mean		1.781	7.756	20.213	20.553
SD		0.8908	1.9330	3.9026	3.8948
CV (%)		50.0	24.9	19.3	18.9
Reference Formulation (A)					
N		8	8	8	8
Mean		2.500	7.127	20.834	21.219
SD		0.8557	1.9579	3.5380	3.6377
CV (%)		34.2	27.5	17.0	17.1
Geometric Mean		2.367	6.907	20.586	20.962
ANOVA p-value					
Untransformed	From	-	0.2631	0.6719	0.6576
	Sequence	-	0.6848	0.7379	0.7663
	Period	-	0.1149	0.4208	0.4928
Ln-transformed	From	-	0.2029	0.6508	0.6440
	Sequence	-	0.6946	0.7528	0.7793
	Period	-	0.1115	0.4300	0.4886
Least Square Means					
Untransformed	Test	-	7.756	20.213	20.553
	Reference	-	7.127	20.834	21.219
Ln-transformed	Test	-	7.555	19.861	20.207
	Reference	-	6.907	20.586	20.962
Ratio of Least Square Means (%)(Test/Reference)					
Untransformed		-	108.8	97.0	96.9
Ln-transformed		-	109.4	96.5	96.4
Intra Subject Variability (%)		-	13.7	13.6	13.7
Ln-transformed		-	12.6	15.1	15.2
90% C.I. –Untransformed					
	Lower	-	94.93	84.01	83.79
	Upper	-	122.72	110.03	109.94
	Power (%)	-	63.1	69.6	69.1
90% C.I. –ln-transformed					
	Lower	-	96.83	83.36	83.26
	Upper	-	123.57	111.67	111.61
	Power (%)	-	84.5	68.9	68.6
Wilcoxon 2-Sample Test p-value (Normal Approx.)		0.1668			

C.I. = Confidence Interval

Table 5.5 :Summary of pharmacokinetic parameters of BID product in fed study

Measures		T_{max}	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$
Test Formulation (B)					
N		8	8	8	8
Mean		3.156	6.469	16.421	17.468
SD		1.4075	2.3465	2.7601	2.6231
CV (%)		44.6	36.3	16.8	15.0
Geometric Mean		2.894	6.070	16.228	17.302
Reference Formulation (A)					
N		8	8	8	8
Mean		2.469	6.963	16.860	18.625
SD		0.8705	1.9999	3.0106	2.5108
CV (%)		35.3	28.7	17.9	13.5
Geometric Mean		2.336	6.640	16.585	18.492
ANOVA p-value					
Untransformed	From	-	0.5329	0.5777	0.0453
	Sequence	-	0.5418	0.2001	0.3426
	Period	-	0.1914	0.7741	0.3895
Ln-transformed	From	-	0.5502	0.7048	0.0480
	Sequence	-	0.5092	0.2025	0.3441
	Period	-	0.1413	09611	0.3060
Least Square Means					
Untransformed	Test	-	6.469	16.421	17.468
	Reference	-	6.963	16.860	18.625
Ln-transformed	Test	-	6.070	16.228	17.302
	Reference	-	6.640	16.585	18.492
Ratio of Least Square Means (%) (Test/Reference)					
Untransformed		-	92.9	97.4	93.8
Ln-transformed		-	91.4	97.8	93.6
Intra Subject Variability (%)					
Untransformed		-	22.2	9.0	5.1
Ln-transformed		-	29.0	11.0	5.4
Wilcoxon 2-Sample Test p-value (Normal Approx.)		0.5205			

As seen from the Tables 5.4 and 5.5, the 90% confidence interval for ln-transformed data in fasted study and ln-transformed T/R ratio in fed study, for Cmax, AUC_{0-t} and $AUC_{0-\infty}$ are between 80-125% as recommended by USFDA guidelines for establishing bioequivalence. Hence the Cefaclor BID formulation developed was bioequivalent to Ceclor CD .

5.3.2 OD matrix

The criteria for development of OD formulations for β-lactum antibiotics are:

a) T>MIC should be greater than 40% of the dosing interval to ensure greater than 90% bacteriological cure.

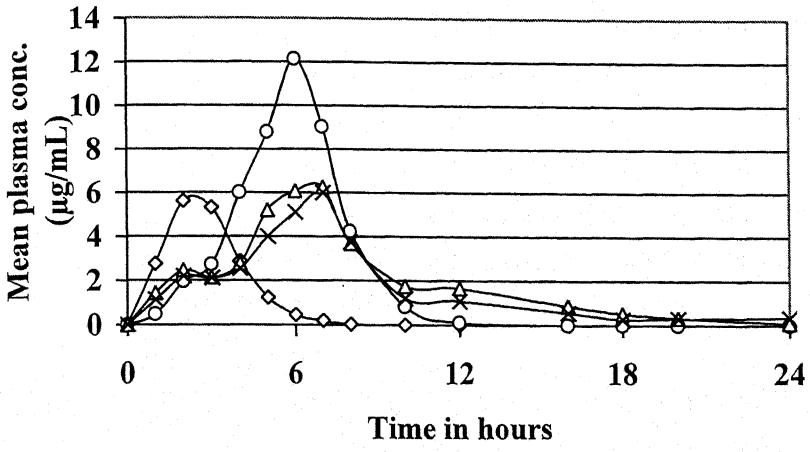
b) The $AUC_{0-\infty}$ of a single dose of the OD formulation should preferably be between 80-120% of that obtained on administering the immediate release formulation for the recommended number of times (TID in this case).

c) C_{max} should be comparable to that of conventional drug product.

d) $t_{1/2}$ (elimination half-life), which is an indicator of potential controlled release system, should be comparable or higher than that of conventional drug product.

It is critical to understand the absorption window and match the release profile with it, in order to maximize bioavailability, yet maintain the maximum possible rate controlled release profile. In case the rate control is beyond the absorption window, one ends up with a sub-optimal bioavailability. Alternatively, if the drug is released well within the absorption window, one ends up with very high C_{max} without providing the necessary extension to achieve the desired T>MIC value. In order to achieve optimum bioavailability, in the present study, three OD formulations, differing in the duration of *in-vitro* release profile were studied. The mean plasma profiles obtained for these formulations and that for the reference product (Distaclor capsules, Eli Lilly) are shown in Fig 5.6

Fig. 5.6 Mean plasma profiles of OD formulations (1500mg) and Distaclor capsules (500mg)



The comparative values for the various pharmacokinetic parameters obtained for the OD formulations and Distaclor capsules are summarized in Table 5.6

Table 5.6 Summary of pharmacokinetic parameters for OD formulations in fed study

Parameter	Distaclor capsules 500mg TID	OD formulation(1500mg) with		
		6 hr profile	10 hr profile	12 hr profile
T>MIC(1µg/mL)	-	35.10	55.125	52.76
*T/R-$AUC_{0-\infty}$	-	85.38	83.50	64.86
**T/R - C_{max}	-	171.93	102.41	91.22
$t_{1/2}$ (h)	.774	0.869	2.526	1.960
T_{max} (h)	2.195	5.75	6.153	6.75

* T/R for AUC is with respect to three capsules of Distaclor 500mg

** T/R for C_{max} is with respect to one capsule of Distaclor 500mg

5.2.3.1 Implications of T>MIC, $AUC_{0-\infty}$ and C_{max}

As seen from the data, the 6 hour profile does not provide adequate extension of the release to cover the absorption window thus resulting in a very high C_{max} without ensuring the desired T>MIC of 40%. On the other hand, the formulation with 12 hour profile extends the release of drug beyond the absorption window. This is evidenced by the fact that although its C_{max} is comparable with that of the conventional product and T>MIC >40%, it fails to meet the criteria for $AUC_{0-\infty}$.

The formulation with 10 hour profile fulfils all the 3 criteria - with a T>MIC >40% and T/R for C_{max} and $AUC_{0-\infty}$ comparable with that of conventional product, demonstrating that the rate control was able to match the absorption window.

5.2.3.2 Implications of $t_{1/2}$

The failure of the 6 hour profile to comply to the T>MIC requirement could be attributed to its $t_{1/2}$ value which is comparable to that of the conventional product i.e. 0.869 Vs 0.774. This means that the drug gets rapidly absorbed and eliminated rather than getting absorbed slowly over a longer time period. The significantly high $t_{1/2}$ value obtained for the 10 hour profile (2.526h) as compared to the conventional product, demonstrates the above postulation and results in prolonged T>MIC.

5.2.3.3 Implications of T_{max}

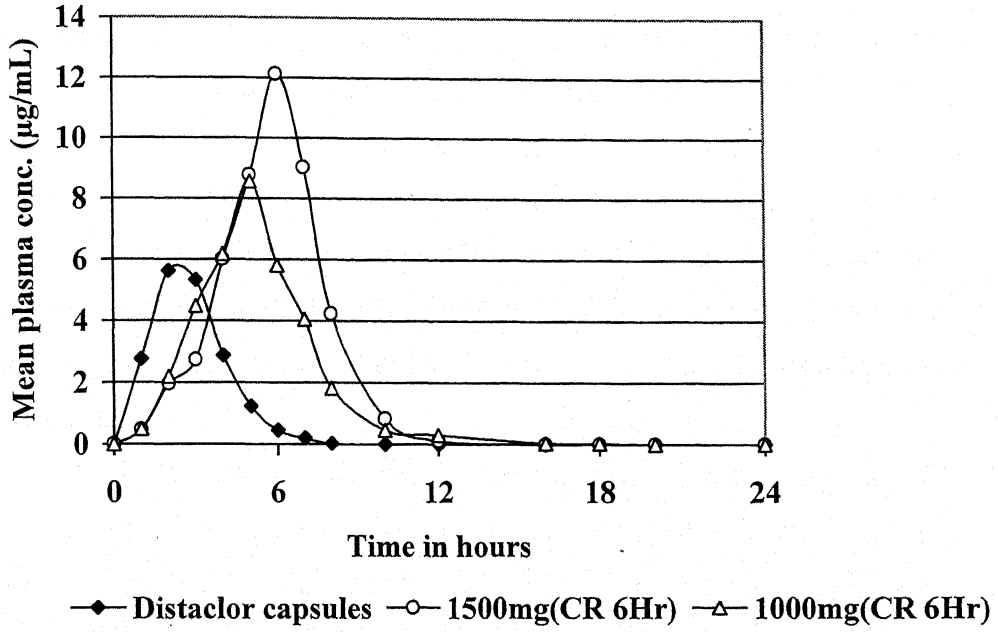
The OD formulations have a longer T_{max} as compared to the conventional capsule which indicates that the matrix is capable of maintaining its integrity and there is less likelihood of dose dumping to take place if properly formulated.

5.3.3 OD formulations with and without Probenecid

Probenecid has often been used concomitantly to enhance the bioavailability of β-lactum antibiotics due to its inherent ability to reduce their excretion. In the present study, use of probenecid has been coupled with a controlled release dosage form of cefaclor so as to reduce the dosing frequency further (BID to OD).

To study if it was possible to lower the dose of Cefaclor in its OD formulation, a 1000mg OD tablet formulation with 6 hour dissolution profile was prepared. The mean plasma profiles obtained for this formulation and that for the reference product (Distaclor capsules, Eli Lilly) and the OD formulation containing 1500mg of drug with 6 hour dissolution profile are shown in Fig 5.7

Fig. 5.7: Plasma profile of OD products of varying doses (1500/1000mg) with 6 hour dissolution profile as compared to Distaclor capsules



The comparative values for the various pharmacokinetic parameters obtained for the OD formulations and Distaclor capsules are summarized in Table 5.7

Table 5.7 Summary of pharmacokinetic parameters for OD products(6-hour profile) containing 1000/1500mg cefaclor in fed study

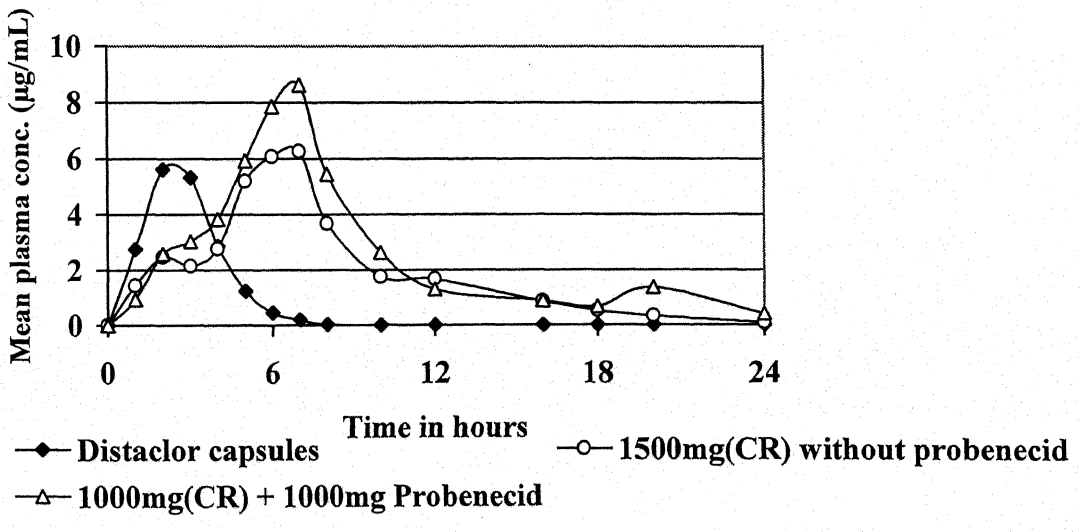
Parameter	Distaclor capsules	6 hour profile OD formulation with cefaclor	
		1000mg	1500mg
T>MIC(1µg/mL)	-	30.60	35.10
*T/R-$AUC_{0-\infty}$	-	66.29	85.38
**T/R - C_{max}	-	135.95	171.93
$t_{1/2}$ (h)	.774	1.277	0.869
Tmax (h)	2.195	4.243	5.75

* T/R for AUC is with respect to three capsules of Distaclor 500mg
** T/R for C_{max} is with respect to one capsule of Distaclor 500mg

The C_{max} obtained with both the formulations was high, although that with the 1500mg dose was higher. The high C_{max} value indicated that the dose is being released too fast and that the profile needs to be further retarded. However, the AUC for the 1000mg dose was much lower than the required 80% indicating that the dose is insufficient to provide bioavailability comparable with that of conventional capsules. Besides the T>MIC (1μg/mL) too was low. This indicated that for the 1000mg dose to work, it was required to improve bioavailability and T>MIC. This could be achieved by either increasing the drug dose to 1500mg coupled with further retarding the release profile upto 10 hours (as discussed in section 5.3.2) OR by retarding the excretion of cefaclor by means of co-administration of probenecid.

The mean plasma profiles obtained for 1000mg dose of 10 hour profile OD product co-administered with 1000mg probenecid, the reference product (Distaclor capsules, Eli Lilly) and the OD formulation containing 1500mg of drug with 10 hour dissolution profile are shown in Fig 5.8

Fig. 5.8: Plasma profile of OD products (10 hr profile) with 1000mg Cefaclor+ 1000mg Probenecid, 1500mg Cefaclor as compared to Distaclor capsules



The comparative values for the various pharmacokinetic parameters obtained for the OD formulations with and without Probenecid and Distaclor capsules are summarized in Table 5.8

Table 5.8 Summary of pharmacokinetic parameters for OD products(10-hour profile) containing 1000mg + probenecid/1500mg cefaclor in fed study

Parameter	**Distaclor capsules**	**10 hour profile OD formulation with cefaclor**	
		1000mg + Probenecid	**1500mg**
T>MIC(1μg/mL)	-	57.375%	55.125%
*T/R-$AUC_{0-\infty}$	-	104.84	83.50
**T/R - C_{max}	-	128.68	102.41
t1/2 (h)	.774	3.791	2.526
Tmax (h)	2.195	6.581	6.153

* T/R for AUC is with respect to three capsules of Distaclor 500mg
** T/R for Cmax is with respect to one capsule of Distaclor 500mg

The C_{max} obtained after co-administration with probenecid is 128.68% which is within the limit of 70-143%, which is the proposed limit for C_{max} as per the European Union guidelines. Besides, the AUC is within 80-120% limit and the T>MIC (1μg/mL) is above the desired 40% indicating that combination of 1000mg of cefaclor along with 1000mg of probenecid fulfills all the requirements for OD dosage form. Use of this combination will reduce the daily dose of cefaclor from 1.5g to 1g thus decreasing cost of therapy.

5.4 *In-vitro- In-vivo* correlationship

5.4.1 Profile duration (*in-vitro*) Vs T_{max} (*in-vivo*)

As can be seen from Table 5.6, as the duration of the *in-vitro* profile increased from 6 hours to 12 hours, the *in-vivo* T_{max} also increases from 5.75 hours in the 6 hour profile to 6.75 hours in the 12 hour profile.

5.4.2 Extent of release (*in-vitro*) and mean time plasma concentration (*in-vivo*)

To study the relationship between *in-vitro* release and *in-vivo* mean plasma concentration, graphs were plotted for each hour as shown in Figs. 5.9-5.14.

Fig. 5.9: Extent released (*in-vitro*) Vs Mean plasma concentration (*in-vivo*) at 1 hour

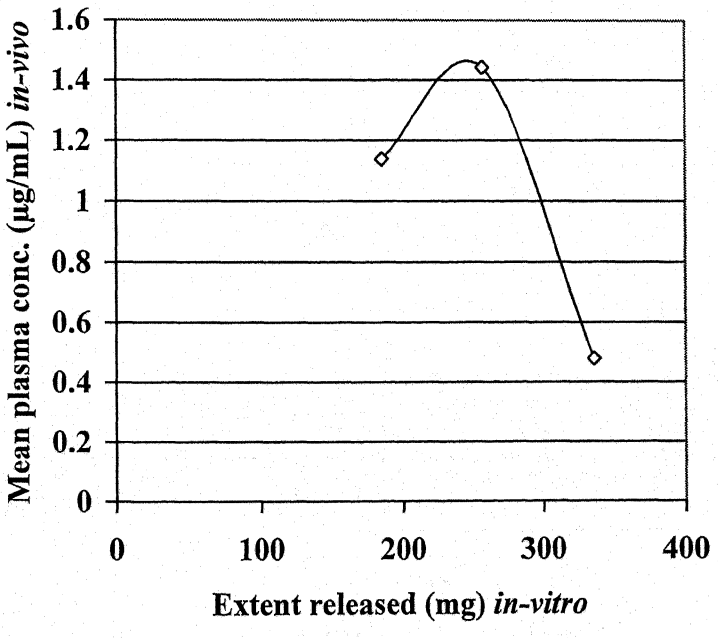


Fig. 5.10: Extent released (*in-vitro*) Vs Mean plasma concentration (*in-vivo*) at 2 hours

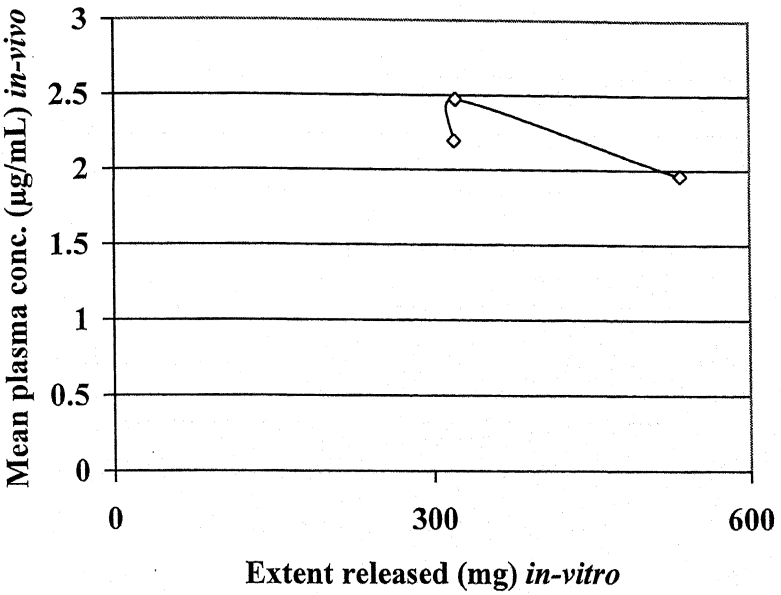


Fig. 5.11: Extent released (*in-vitro*) Vs Mean plasma concentration (*in-vivo*) at 3 hours

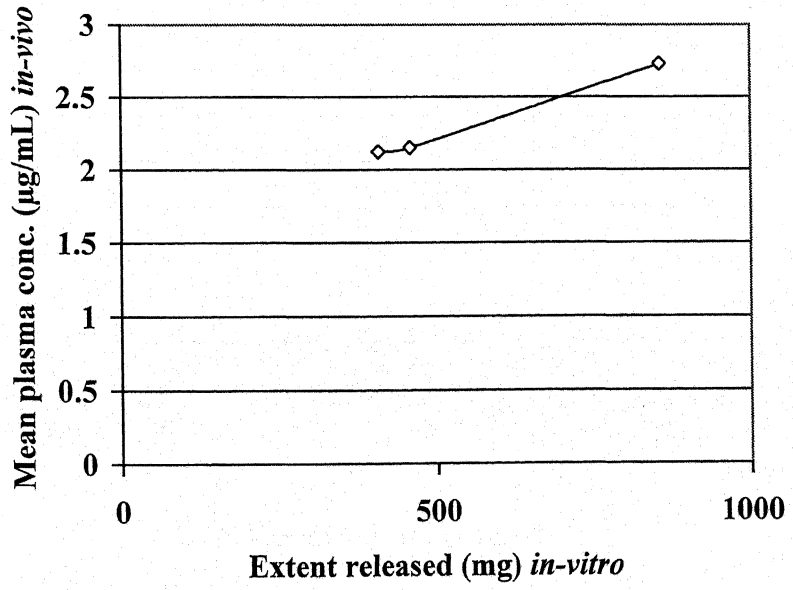


Fig. 5.12: Extent released (*in-vitro*) Vs Mean plasma concentration (*in-vivo*) at 4 hours

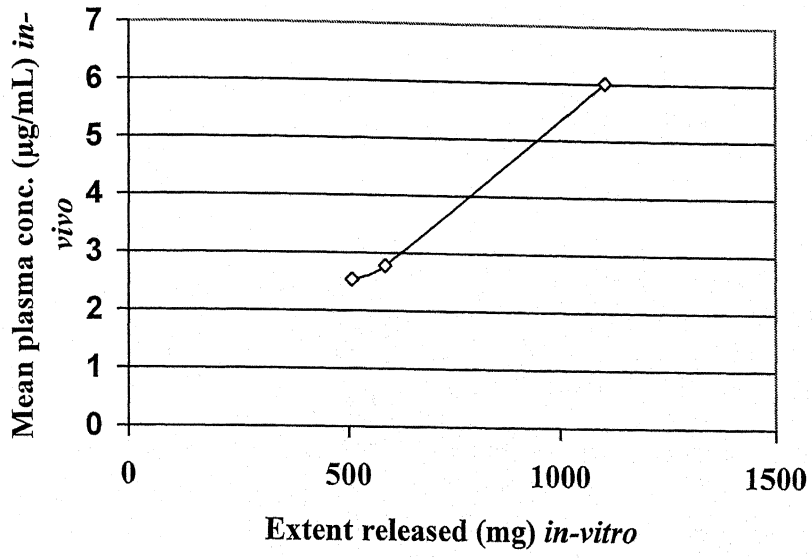


Fig. 5.13: Extent released (*in-vitro*) Vs Mean plasma concentration (*in-vivo*) at 5 hours

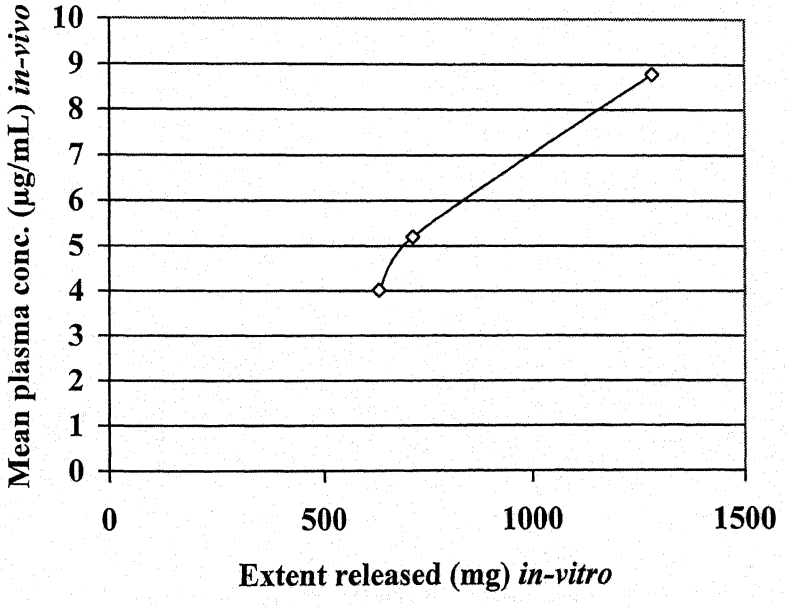
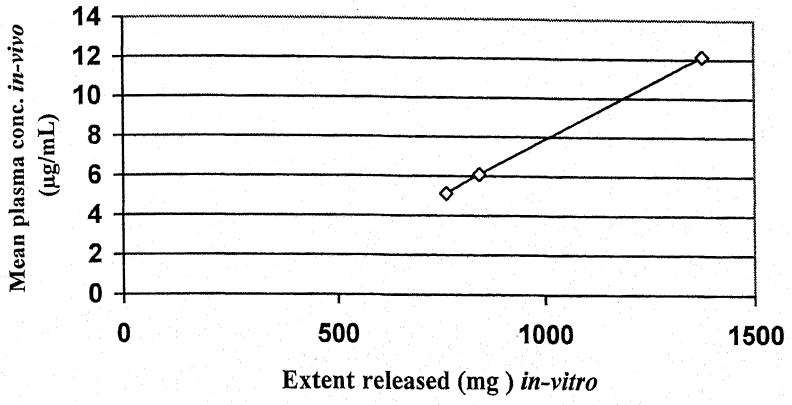


Fig. 5.14: Extent released (*in-vitro*) Vs Mean plasma concentration (*in-vivo*) at 6 hours

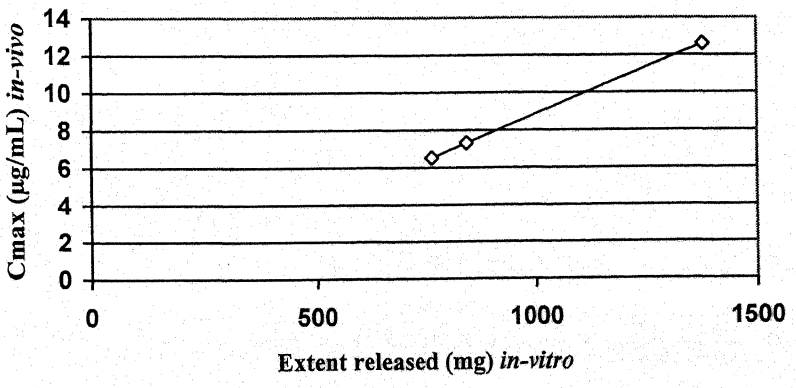


It is evident from Fig. 5..11-5.14 that a perfect correlation (r^2 = 0.99) between the extent released *in-vitro* and *in-vivo* mean time plasma concentration exists during 3-6hours period i.e when the dissolution medium used for studying *in-vitro* release is pH 6.8 phosphate buffer.

5.4.3 Extent of release(*in-vitro*) Vs C_{max} (*in-vivo*)

Since the Tmax of all the OD formulations studied, were in the range of 5.75-6.75 hours, the relationship between the amount released at 6 hours and C_{max} was studied by plotting a graph between them as shown in Fig. 5.15. A perfect correlation (r^2=0.99) was also obtained on plotting C_{max} Vs the extent released in 6 hours.

Fig. 5.15: Extent released *in-vitro* at 6 hours Vs C_{max} *in-vivo*



6

CONCLUSION

The following conclusions can be drawn from these studies:

6.1 OCRS-BID bioequivalent to Ceclor CD 500mg

6.1.1 A patentable twice-a-day formulation has been developed using xanthan gum and sodium alginate as rate controlling polymers. The product has been stabilized using calcium sulphate and hydroxypropyl methylcellulose phthalate. The prototype formula is shown in Table 3.21 and the process thereunder.

6.1.2 The proposed specifications for the product and the standard testing procedure is enclosed as Annexure 14.

6.1.3 Based on the satisfactory data, after 3 months at the accelerated storage condition, a 24 month shelf life can be assigned to the product in Aluminum strip pack (Annexure 3).

6.1.4 The product is bioequivalent with Ceclor CD of Eli Lilly, USA in fed and fasted conditions in terms of C_{max} and AUC (Tables 5.4 & 5.5).

6.2 OCRS-intervention of conventional 500mg TID cefaclor for a bioavailable and stable, 1.0g, once-a-day preparation with probenecid

A bioavailable once-a-day intervention was achieved by decreasing the dose of the drug substance from 1.5g to 1.0g by co-administering with 1g of probenecid. A 10-hour profile *in-vitro*, showed desirable T>MIC, C_{max} and AUC *in-vivo*.

6.3 OCRS intervention of conventional 500mg TID cefaclor for a bioavailable and stable, 1.5g, once-a-day preparation

6.3.1 A patentable OD formulation of Cefaclor has been developed using xanthan gum and sodium alginate as rate-controlling polymers. The product has been stabilized using calcium sulphate and hydroxypropyl methylcellulose phthalate. The prototype formula is shown in Table 4.14.

6.3.2 The proposed specifications for the product and the standard testing procedure are enclosed as Annexure 15.

6.3.3 Based on the satisfactory data, after 3 months at the accelerated storage condition, a 24 month shelf life can be assigned to the product in Aluminum strip pack (Annexure 11).

6.3.4 Following a number of bioavailability studies, we were able to establish an OD intervention of an existing TID/BID preparation with a desired *in-vitro* and *in-vivo* profile. It was established that a 10 hour release profile, offered an optimal extension matching the absorption window thereby maximizing T>MIC (1μg/mL) and resulting in T/R of AUC and C_{max} between 80-120% of that obtained on administering 500mg conventional capsules three times a day in fed healthy human volunteers. The $t_{1/2}$ of the OD product was extended beyond that of the conventional product. (Table5.6). This satisfies the four main criteria for an OD formulation.

6.3.5 The OD matrix systems developed show a zero-order release profile and exhibit anomalous behavior.

6.3.6 A perfect (r^2=0.99) correlation has been established between amount of drug released *in-vitro* and drug plasma concentration *in-vivo*.

6.3.7 In view of the large number of variables like pH-dependent solubility, permeability, absorption window, $t_{1/2}$ etc while designing a controlled release system, the strategy and value of zero-order release and/or *in-vitro in-vivo* correlation is yet to be established. Large number of such studies, need to be conducted, using drug substances with aforementioned variables to draw a meaningful conclusion.

7

PATENTS AND PUBLICATIONS

PATENTS

1 WO 02/41876 A1

Title: Pharmaceutical composition for controlled release of an active ingredient

Applicant: Lupin Laboratories Limited

Inventors: Sen Himadri, Kshirsagar Rajesh S, Kandi Chandrashekhar S & Bhamare Shailesh

2 FPAA/147(PCT)

Title: An improved stable pharmaceutical composition for controlled release of an active ingredient

Applicant: Lupin Limited

Inventors: Sen Himadri, Kshirsagar Rajesh S & Bhamare Shailesh S

PUBLICATIONS

1 A stable, bioavailable once daily controlled release intervention of Cefaclor.

Authors: Authors: Sen Himadri, Bhamare Shailesh S, Arora Sudershan & Chandra Ramesh

Communicated with : Drug Development and Industrial Pharmacy

2 A stable, bioavailable twice daily controlled release intervention of Cefaclor.

Authors: Sen Himadri, Bhamare Shailesh S, Arora Sudershan & Chandra Ramesh

Communicated with : Indian Journal of Pharmaceutical Sciences

8

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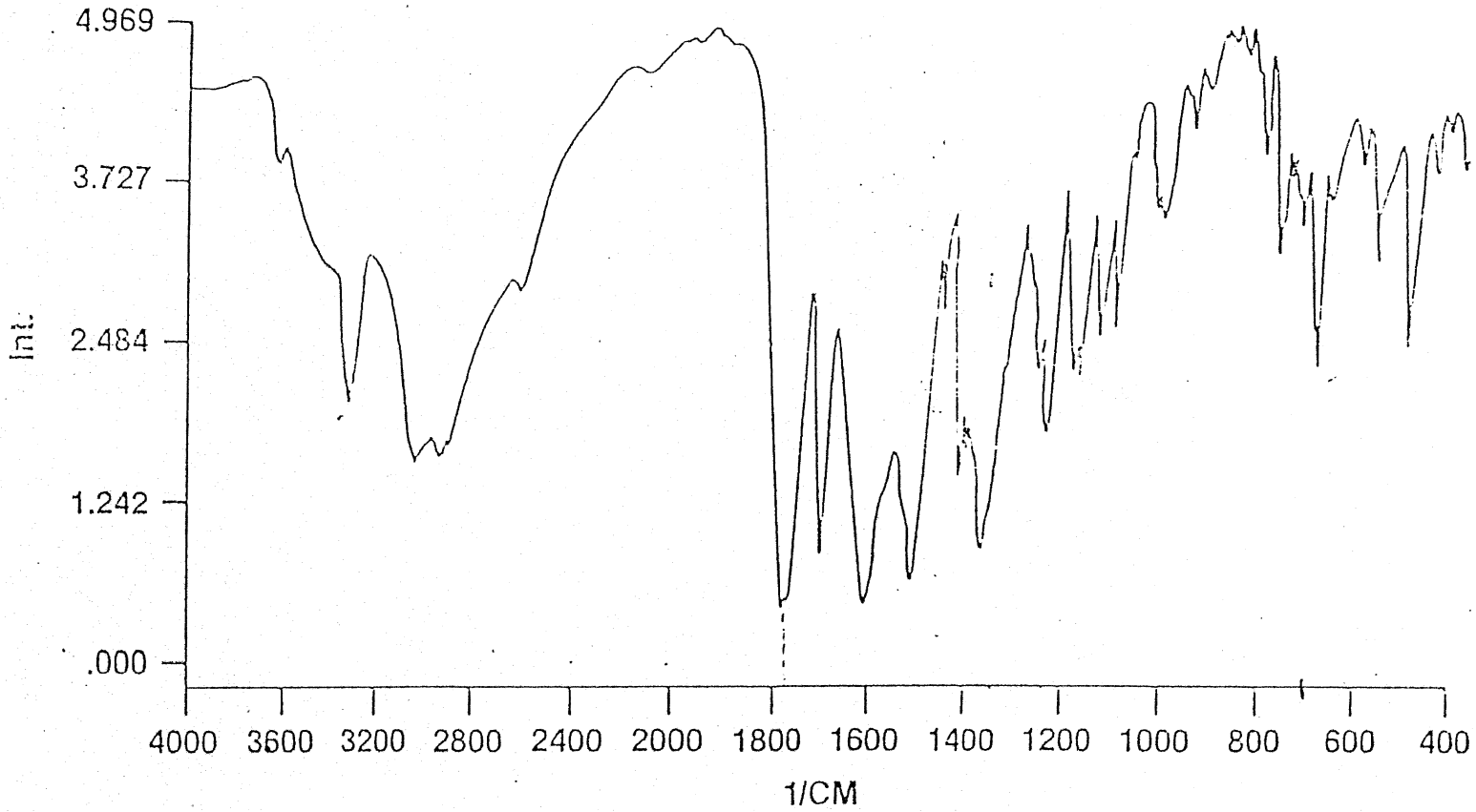
ANNEXURES

Annexures 1 & 2

X-RAY POWDER DIFFRACTION AND INFRARED SPECTURM (IN KBr PELLET) OF CEFACLOR MONOHYDRATE

Annexure 1
X-Ray Powder Diffraction Data of Cefaclor Monohydrate
λ=1.5418

"d" value (A)	Intensities (I/Io)
12.90	0.75
10.05	0.17
6.58	0.13
6.08	0.13
5.42	0.96
5.01	1.00
4.75	0.04
4.06	0.54
3.86	0.04
3.69	0.29
3.53	0.58
3.41	0.04
3.29	0.17
3.23	0.13
3.13	0.04
2.99	0.21
2.81	0.25
2.67	0.08
2.52	0.08
2.48	0.04
2.35	0.17
2.26	0.17
2.15	0.04
2.07	0.08
1.99	0.21
1.94	0.08



The Infrared Spectrum of Cefaclor in a KBr Pellet

Annexure 3

STABILITY DATA OF CEFACLOR BID FORMULATION IN ALUMINUM STRIP FOR 3 BATCHES

Annexure 3
Accelerated Stability Data

Product Name : Cefaclor BID

Strength : 500 mg

Batch No. : CSK(3)137

Pack : Aluminum strip

Description : Blue colour, film coated, biconvex, capsules shape tablets

Storage condition and Storage Time (Months)	**Parameter**						
	Description	**Water (%)**	**Assay (%)**	**Dissolution profile (% drug release)**			
				1hr (%)	**2hrs (%)**	**3hrs (%)**	**4hrs (%)**
Limits	As above	NMT 7.0%	90-110%	15.0-55.0	40.0-80.0	60.0-95.0	NLT 85
Initial	Complies	5.7	101.4	34.1	58.3	79.6	91.3
40°C /75%RH							
1 month	Complies	5.8	100.2	42.6	61.0	80.9	94.9
2 months	Complies	5.85	98.4	46.1	70.2	92.1	97.0
3 months	Complies	5.95	97.5	48.9	73.8	92.5	96.0
25°C /60%RH							
3 months	Complies	5.75	100.2	45.0	64.0	82.0	91.0

Annexure 3 (Contd)
Accelerated Stability Data

Product Name : Cefaclor BID

Strength : 500 mg

Batch No. : CSK(46)23A

Pack : Aluminum strip

Description : Blue colour, film coated, biconvex, capsules shape tablets

Storage condition and Storage Time (Months)	**Parameter**						
	Description	**Water (%)**	**Assay (%)**	**Dissolution profile (% drug release)**			
				1hrs (%)	2hrs (%)	3hrs (%)	4hrs (%)
Limits	As above	NMT 7.0%	90-110%	15.0-55.0	40.0-80.0	60.0-95.0	NLT85
Initial	Complies	5.8	100.2	28.5	57.2	86.1	95.6
40°C /75%RH							
1 month	Complies	5.7	100.1	44.3	67.4	87.2	95.6
2 months	Complies	5.9	99.8	38.3	65.9	76.9	90.8
3 months	Complies	6.0	99.7	44.4	70.1	83.2	92.8
25°C /60%RH							
3 months	Complies	5.7	99.6	42.0	57.0	75.8	92.0

Annexure 3 (Contd)
Accelerated Stability Data

Product Name : Cefaclor BID

Strength : 500 mg

Batch No. : CSK(46)27

Pack : Aluminum strip

Description : Blue colour, film coated, biconvex, capsules shape tablets

Storage condition and Storage Time (Months)	**Parameter**						
	Description	**Water (%)**	**Assay (%)**	**Dissolution profile (% drug release)**			
				1hrs (%)	2hrs (%)	3hrs (%)	4hrs (%)
Limits	As above	NMT 7.0%	90-110%	15.0-55.0	40.0-80.0	60.0-95.0	NLT85
Initial	Complies	5.8	100.2	25.0	50.1	75.2	92.5
40°C /75%RH							
1 month	Complies	5.7	99.3	30.2	55.8	81.2	94.4
2 months	Complies	5.9	98.4	32.4	58.4	83.2	95.2
3 months	Complies	5.95	97.4	34.8	58.7	82.1	92.8
25°C /60%RH							
3 months	Complies	5.75	99.2	26.3	52.1	77.8	93.1

Annexures 4 & 5

PHARMACOKINETIC DATA OF CEFACLOR BID Vs CECLOR CD UNDER FASTED & FED CONDITIONS

Annexure 4

Individual and mean plasma concentrations (µg/mL) for Ceclor CD (reference) in fasted volunteers

Subject	Time (h)								
	0.00	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.50
1	0.000	0.282	1.243	5.511	5.581	5.010	5.146	6.297	6.952
2	0.000	0.194	1.477	3.305	3.023	3.444	5.616	9.090	7.918
3	0.000	2.111	1.980	3.636	4.197	5.174	5.201	5.010	5.059
4	0.000	1.407	3.469	5.527	7.871	6.643	5.902	5.740	5.696
5	0.000	0.200	1.470	2.505	2.838	3.621	3.805	3.917	9.227
6	0.000	0.375	0.715	0.770	1.094	1.859	2.163	2.099	6.489
7	0.000	3.269	2.827	2.657	2.354	2.464	2.809	3.353	4.197
8	0.000	1.136	3.247	3.144	4.801	4.308	4.975	5.016	4.472
N	8	8	8	8	8	8	8	8	8
Mean	0.0000	1.1218	2.0533	3.3820	3.9701	4.0654	4.4523	5.0653	6.2514
SE	0.00000	0.39293	0.35735	0.55711	0.75129	0.54832	0.48424	0.74674	0.61667

Subject	3.0	4.00	6.00	8.00	10.00	T_{max}	C_{max}	AUClast
1	4.685	1.645	0.290	0.000	0.000	2.500	6.952	17.836
2	7.324	4.630	0.910	0.284	0.195	2.000	9.090	26.678
3	4.555	3.250	0.464	0.000	0.000	1.750	5.201	19.002
4	4.710	1.883	0.295	0.000	0.000	1.250	7.871	19.533
5	10.615	4.598	0.761	0.000	0.000	3.000	10.615	25.337
6	5.703	3.328	0.889	0.191	0.000	2.500	6.489	17.061
7	5.659	3.429	0.817	0.176	0.000	3.000	5.659	19.058
8	4.705	5.137	0.574	0.120	0.000	4.000	5.137	22.163
N	8	8	8	8	8	8	8	8
Mean	5.9943	3.4875	0.6251	0.0964	0.0244	2.5000	7.1266	20.8336
SE	0.73692	0.44953	0.09025	0.03966	0.02441	0.30252	0.69224	1.25086

Annexure 4

Individual and mean plasma concentrations(µg/mL) for Cefaclor BID (test) tablets in fasted volunteers

Subject	Time (h)								
	0.00	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.50
1	0.000	5.130	7.295	6.943	7.489	7.516	8.325	8.762	6.326
2	0.000	8.016	11.244	10.153	11.151	10.585	8.812	6.417	4.293
3	0.000	3.913	4.039	3.418	3.611	4.070	4.539	4.798	5.889
4	0.000	1.152	3.760	7.843	7.633	8.064	9.289	8.152	6.480
5	0.000	4.203	6.082	5.555	6.114	6.702	7.451	7.203	7.866
6	0.000	0.501	0.626	0.735	1.131	1.220	2.096	2.369	5.317
7	0.000	6.791	5.601	5.208	4.374	4.144	3.858	3.715	5.485
8	0.000	3.409	3.998	5.853	6.703	5.869	4.434	3.384	3.078
N	8	8	8	8	8	8	8	8	8
Mean	0.0000	4.1397	5.3306	5.7134	6.0259	6.0211	6.1006	5.6001	5.5984
SE	0.00000	0.90425	1.09793	1.00097	1.06886	1.01865	0.95034	0.83778	0.51155

Subject	3.0	4.00	6.00	8.00	10.00	T_{max}	C_{max}	AUClast
1	3.623	1.444	0.246	0.000	0.000	2.000	8.762	22.893
2	2.748	1.206	0.217	0.000	0.000	0.750	11.244	24.632
3	4.521	1.451	0.303	0.000	0.000	2.500	5.889	17.002
4	3.979	1.402	0.353	0.000	0.000	1.750	9.289	21.317
5	5.935	2.423	0.256	0.000	0.000	2.500	7.866	24.528
6	5.500	3.847	0.904	0.236	0.000	3.000	5.500	17.153
7	4.566	2.641	0.454	0.000	0.000	0.500	6.791	20.319
8	2.083	0.868	0.194	0.000	0.000	1.250	6.703	13.859
N	8	8	8	8	8	8	8	8
Mean	4.1194	1.9103	0.3659	0.0295	0.0000	1.7813	7.7556	20.2128
SE	0.46010	0.34893	0.08227	0.02954	0.00000	0.31495	0.68340	1.37978

Annexure 4

Results of Bioequivalence study of Ceclor CD (500 mg) (reference) and Cefaclor BID (test) tablets in fasted volunteers

Subject	T_{max} (h)		C_{max} (µg/mL)		AUC_{0-t}(µg*h/mL)		$AUC_{0-\infty}$(µg*/h/mL)		AUC% Extrap	
	A	B	A	B	A	B	A	B	A	B
1	2.50	2.00	6.95	8.76	17.84	22.89	18.15	23.17	1.74	1.18
2	2.00	0.75	9.09	11.24	26.68	24.63	27.04	24.89	1.34	1.03
3	1.75	2.50	5.20	5.89	19.00	17.00	19.59	17.35	2.99	2.01
4	1.25	1.75	7.87	9.29	19.53	21.32	19.85	21.76	1.61	2.03
5	3.00	2.50	10.61	7.87	25.34	24.53	26.20	24.77	3.30	0.97
6	2.50	3.00	6.49	5.50	17.06	17.15	17.33	17.49	1.54	1.94
7	3.00	0.50	5.66	6.79	19.06	20.32	19.30	20.90	1.23	2.76
8	4.00	1.25	5.14	6.70	22.16	13.86	22.29	14.10	0.57	1.74
N	8	8	8	8	8	8	8	8	8	8
Mean	2.500	1.781	7.127	7.756	20.834	20.213	21.219	20.553	1.792	1.709
SD	0.8557	0.8908	1.9579	1.9330	3.5380	3.9026	3.6377	3.8948	0.9108	0.6140
Min	1.25	0.50	5.14	5.50	17.06	13.86	17.33	14.10	0.57	0.97
Median	2.500	1.875	6.72	7.33	19.296	20.818	19.721	21.328	1.575	1.840
Max	4.00	3.00	10.61	11.24	26.68	24.63	27.04	24.89	3.30	2.76
CV%	34.2	50.0	27.5	24.9	17.0	19.3	17.1	18.9	50.8	35.9
Geometric Mean	2.367	1.535	6.907	7.555	20.586	19.861	20.962	20.207	1.589	1.611

A = Reference **B** = Test

Annexure 4

Individual and mean pharmacokinetic parameters for Cefaclor BID formulations

Subject	λ_z(/hour)		λ_z_lower(/hour)		λ_z_upper (/hour)		$t_{1/2}$(h)	
	A	B	A	B	A	B	A	B
1	0.916	0.895	2.050	3.00	6.00	6.00	0.76	0.77
2	0.538	0.847	2.00	2.00	10.00	6.00	1.29	0.82
3	0.792	0.869	3.00	2.50	6.00	6.00	0.88	0.80
4	0.924	0.799	3.00	1.75	6.00	6.00	0.75	0.87
5	0.881	1.059	3.00	3.00	6.00	6.00	0.79	0.65
6	0.715	0.697	4.00	4.00	8.00	8.00	0.97	0.99
7	0.742	0.786	4.00	3.00	8.00	6.00	0.93	0.88
8	0.939	0.791	4.00	2.50	8.00	6.00	0.74	0.88
N	8	8	8	8	8	8	8	8
Mean	0.8058	0.8431	3.188	2.719	7.250	6.250	0.888	0.833
SD	0.13836	0.10647	0.7530	0.7000	1.4880	0.7071	0.1846	0.0987
Min	0.538	0.697	2.00	1.75	6.00	6.00	0.74	0.65
Median	0.8364	0.8234	3.000	2.750	7.000	6.000	0.831	0.843
Max	0.939	1.059	4.00	4.00	10.00	8.00	1.29	0.99
CV%	17.2	12.6	23.6	25.7	20.5	11.3	20.8	11.9
Geometric Mean	0.7941	0.8375	3.105	2.640	7.124	6.220	0.873	0.828

A = Reference B = Test

Annexure 5

Individual and mean plasma concentrations (µg/mL) for Ceclor CD (reference) in fed volunteers

		Concentration (µg/mL)													
Time → **Subject ↓**	**0**	**0.5**	**0.75**	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.5**	**3**	**3.5**	**4**	**6**	**8**	**10**
1	0.00	2.49	5.55	6.72	7.17	8.84	7.38	7.28	5.22	2.40	1.44	0.71	0.00	0.00	0.00
2	0.00	0.00	0.31	0.44	0.55	0.89	1.80	4.07	7.57	6.09	4.66	2.85	0.56	0.00	0.00
3	0.00	0.00	0.21	0.32	0.40	0.44	1.34	3.02	3.13	1.91	1.94	3.13	1.36	0.00	0.00
4	0.00	0.00	4.43	6.79	7.62	7.53	8.65	6.49	4.30	2.13	2.03	2.07	0.26	0.00	0.00
5	0.00	0.00	0.00	0.78	2.20	5.35	4.16	4.36	4.94	8.77	3.61	1.88	0.23	0.00	0.00
6	0.00	0.00	0.79	2.79	5.12	6.72	6.44	6.35	4.41	4.63	4.91	3.75	1.53	0.00	0.00
7	0.00	0.37	1.09	2.44	2.95	4.53	4.34	3.34	4.11	5.15	4.95	4.17	0.52	0.00	0.00
8	0.00	0.35	0.81	1.22	1.13	1.02	1.46	1.79	6.89	3.19	4.64	6.06	0.73	0.00	0.00
N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Mean	0.000	0.402	1.647	2.687	3.392	4.414	4.445	4.590	5.071	4.283	3.524	3.080	0.648	0.000	0.000
SD	0.0000	0.8610	2.1150	2.6620	0.9010	3.2770	2.8250	1.9320	1.4790	2.3680	1.4910	1.6400	0.5420	0.0000	0.0000
Min	0.00	0.00	0.00	0.32	0.40	0.44	1.34	1.79	3.13	1.91	1.44	0.71	0.00	0.00	0.00
Max	0.00	2.49	5.55	6.79	7.62	8.84	8.65	7.28	7.57	8.77	4.95	6.09	1.53	0.00	0.00
CV%	-	214.0	128.4	99.1	85.5	74.3	63.5	42.1	29.2	55.3	42.3	53.3	83.7	-	-

Annexure 5

Individual and mean plasma concentrations (µg/mL) for Cefaclor BID (test) in fed volunteers

	Concentration (µg/mL)														
Time → **Subject ↓**	**0**	**0.5**	**0.75**	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.5**	**3**	**3.5**	**4**	**6**	**8**	**10**
1	0.00	0.27	1.41	4.81	9.81	8.16	6.54	4.44	3.09	2.67	3.48	2.34	0.36	0.00	0.00
2	0.00	0.00	0.43	0.84	0.98	0.80	0.88	2.39	3.15	2.16	1.60	2.23	2.01	0.62	0.51
3	0.00	0.00	0.48	0.91	1.21	4.21	1.93	2.47	5.01	6.24	4.04	2.27	0.38	0.00	0.00
4	0.00	0.00	0.46	0.78	1.08	0.97	1.26	4.20	9.13	4.05	4.66	2.97	0.97	0.00	0.00
5	0.00	1.65	1.59	1.48	2.47	2.88	5.08	6.88	7.38	3.59	2.09	1.33	0.00	0.00	0.00
6	0.00	0.61	2.19	2.67	2.67	1.91	2.10	2.59	3.80	2.48	2.74	4.33	3.60	0.43	0.00
7	0.00	0.00	0.29	0.51	1.20	1.35	1.21	0.89	0.98	1.10	1.54	4.46	4.62	0.31	0.00
8	0.00	0.00	0.37	0.45	0.54	0.54	0.54	1.02	2.67	4.10	7.10	5.68	1.34	0.00	0.00
N	8		8	8	8	8	8	8	8	8	8	8	8	8	8
Mean	0.000	0.315	0.903	1.554	2.495	2.602	2.442	3.111	4.400	3.299	3.406	3.201	1.661	0.169	0.063
SD	0.0000	0.5800	0.7220	1.4990	3.0460	2.5580	2.1740	1.9900	2.6720	1.5630	1.8730	1.4680	1.6610	0.2490	0.1790
Min	0.00	0.00	0.29	0.45	0.54	0.54	0.54	0.89	0.98	1.10	1.54	1.33	0.00	0.00	0.00
Max	0.00	1.65	2.19	4.81	9.81	8.16	6.54	6.88	9.13	6.24	7.10	5.68	4.62	0.62	0.51
CV%	-	183.9	79.9	96.5	122.1	98.3	89.0	64.0	60.7	47.4	55.0	45.9	100.0	146.9	282.8

Annexure 5

Results of Bioequivalence study of Ceclor CD (500 mg) (reference) and Cefaclor BID (test) tablets in fed volunteers

Subject	T_{max} (h)		C_{max} (µg/mL)		AUC_{0-t} (µg*h/mL)		$AUC_{0-\infty}$ (µg*h/mL)		λ_z (l/h)		λ_z_lower (h)		λ_z_upper (h)		$t_{½}$ (h)		AUC_% Extrap	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	1.500	1.250	8.840	9.810	17.290	17.350	17.860	17.730	1.227	0.920	3.000	3.500	4.000	6.000	0.570	0.750	3.220	2.180
2	2.500	2.500	7.570	3.150	15.810	13.890	16.470	15.360	0.842	0.345	3.500	6.000	6.000	10.000	0.820	2.010	4.000	9.570
3	4.000	3.000	3.130	6.240	10.570	13.980	18.180	14.390	1.178	0.926	2.500	3.500	6.000	6.000	3.890	0.750	41.900	2.880
4	1.750	2.500	8.650	9.130	18.260	16.310	18.550	17.910	0.891	0.608	3.500	3.500	6.000	6.000	0.780	1.140	1.550	8.910
5	3.000	2.500	8.770	7.380	16.000	13.430	16.210	14.780	1.085	0.990	3.500	3.000	6.000	4.000	0.640	0.700	1.310	9.110
6	1.500	4.000	6.720	4.330	21.030	21.640	24.360	22.380	0.461	0.579	3.500	4.000	6.000	8.000	1.500	1.200	13.640	3.290
7	3.000	6.000	5.150	4.620	18.010	18.400	18.570	18.850	0.938	0.670	3.500	4.000	6.000	8.000	0.740	1.030	3.010	2.420
8	2.500	3.500	6.800	7.100	17.910	16.360	18.800	18.330	0.829	0.682	3.500	3.500	6.000	6.000	0.840	1.020	4.710	10.750
N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Mean	2.469	3.156	6.963	6.469	16.860	16.421	18.625	17.468	0.806	0.715	3.313	3.875	5.750	6.750	1.222	1.075	9.167	6.139
SD	0.8705	1.4075	1.9999	2.3465	3.0106	2.7601	2.5108	2.6231	0.3369	0.2180	0.3720	0.9161	0.7071	1.8323	1.1168	0.4226	13.7879	3.7360
Min	1.50	1.25	3.13	3.15	10.57	13.43	16.21	14.39	0.18	0.35	2.50	3.00	4.00	4.00	0.57	0.70	1.31	2.18
Median	2.50	2.75	7.23	6.67	17.60	16.34	18.37	17.82	0.87	0.68	3.50	3.50	6.00	6.00	0.80	1.03	3.61	6.10
Max	4.00	6.00	8.84	9.81	21.03	21.64	24.63	22.38	1.23	0.99	3.50	6.00	6.00	10.00	3.89	2.01	41.90	10.75
CV%	35.3	44.6	28.7	36.3	17.9	16.8	13.5	15.0	41.8	30.5	11.2	23.6	12.3	27.1	91.4	39.3	150.4	60.9
Geometric Mean	2.336	2.894	6.640	6.070	16.585	16.228	18.492	17.302	0.709	0.682	3.292	3.797	5.703	6.533	0.978	1.016	4.633	5.043

A = Reference **B** = Test

Annexure 6

STABILITY DATA OF CEFACLOR OD (750mg) –12 HOUR PROFILE

Annexure 6

Accelerated Stability Data

Product Name : **Cefaclor OD**
Strength : 750mg
Batch No. : CSK(46)127
Description : Blue film coated, capsule shaped, biconvex tablets

Pack	Storage Time (Months)	Parameter										
		Description	Water (%)	Assay (%)	Dissolution profile (% drug released)							
					1 hrs	2 hrs	3 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs
	Limits	As above	NMT 10%	90-110%	To be monitored							
	Initial	Complies	4.72	98.96	11.5	22.6	26.4	32.0	44.2	57.7	75.7	86.7
PVdC (90gsm) coated PVC	**40°C /75%RH**											
	1 month	Complies	5.57	97.23	17.0	31.5	41.4	57.8	87.0	-	-	-
HDPE	**40°C /75%RH**											
	1 month	Complies	5.75	96.97	18.3	33.5	44.0	62.1	88.8	-	-	-

Note: Study was discontinued after one month

Annexure 7

PHARMACOKINETIC DATA OF CEFACLOR OD (2x 750mg) –12 HOUR PROFILE

Annexure 7

Individual and Mean Plasma Concentrations of Cefaclor after Administration of 2*750 mg Cefaclor OD Tablets(12 hr profile) under Fed Condition

Time (h) → Subject ↓	Concentration (µg/mL)														
	0.00	**1.00**	**2.00**	**3.00**	**4.00**	**5.00**	**6.00**	**7.00**	**8.00**	**10.00**	**12.00**	**16.00**	**18.00**	**20.00**	**24.00**
1	0.00	2.39	3.14	2.43	5.45	4.59	5.19	7.96	3.40	0.57	0.00	0.00	0.00	0.00	0.00
2	0.00	0.56	1.60	2.08	2.22	6.77	6.57	7.75	4.23	2.11	1.26	1.05	0.22	0.00	0.00
3	0.00	0.00	2.19	1.69	1.28	2.26	3.91	4.56	3.22	1.74	2.55	1.03	0.53	0.62	0.00
4	0.00	1.08	1.46	1.27	2.02	3.23	4.34	5.59	8.33	2.05	3.23	0.86	0.00	0.00	0.00
5	0.00	0.78	1.98	2.88	2.48	3.91	4.45	6.00	3.22	1.21	1.63	1.56	1.50	1.87	2.79
6	0.00	1.49	1.61	2.20	2.84	5.24	4.82	4.37	1.29	0.41	0.00	0.00	0.00	0.00	0.00
7	0.00	1.85	2.09	1.92	0.99	2.89	7.31	6.78	3.00	0.92	0.00	0.00	0.00	0.00	0.00
8	0.00	0.97	3.47	2.51	2.98	3.16	4.22	5.05	3.25	0.63	0.00	0.00	0.00	0.00	0.00
N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Mean	0.000	1.138	2.193	2.122	2.532	4.008	5.102	6.007	3.741	1.204	1.084	0.562	0.281	0.311	0.349
SD	0.0000	0.7550	0.7380	0.5040	1.3670	1.4710	1.2140	1.3780	2.0260	0.6830	1.2980	0.6330	0.5280	0.6650	0.9860
Minimum	0.00	0.00	1.46	1.27	0.99	2.26	3.91	4.37	1.29	0.41	0.00	0.00	0.00	0.00	0.00
Median	0.000	1.025	2.038	2.138	2.349	3.570	4.636	5.796	3.235	1.067	0.630	0.432	0.000	0.000	0.000
Maximum	0.00	2.39	3.47	2.88	5.45	6.77	7.31	7.96	8.33	2.11	3.23	1.56	1.50	1.87	2.79
CV%	0.00	66.4	33.6	23.7	54.0	36.7	23.8	22.9	54.2	56.7	119.8	112.6	187.8	214.2	282.8
Geometric Mean	-	-	2.095	2.065	2.248	3.790	4.988	5.870	3.343	1.025	-	-	-	-	-

Annexure 7

Individual and Mean Pharmacokinetic Parameters of Cefaclor after Administration of 2*750 mg Cefaclor OD Tablets (12 hr profile) under Fed Condition

Subject	Tmax (h)	Cmax (µg/mL)	AUC $_{0-t}$ (µg.h/mL)	AUC $_{0-\infty}$ (µg.h/mL)	AUC_% Extrap	λ_z (1/h)	λ_z_lower (h)	λ_z_upper (h)	$t_{½}\lambda$ (h)
1	7.00	7.96	36.82	37.47	1.74	0.879	7.00	10.00	0.79
2	7.00	7.75	45.22	46.04	1.77	0.268	7.00	18.00	2.59
3	7.00	4.56	36.60	40.55	9.76	0.156	7.00	20.00	4.44
4	8.00	8.33	47.01	50.53	6.97	0.245	8.00	16.00	2.83
5	7.00	6.00	53.49	*	*	*	*	*	*
6	5.00	5.24	24.91	25.52	2.38	0.666	6.00	10.00	1.04
7	6.00	7.31	29.26	30.67	4.59	0.655	7.00	10.00	1.06
8	7.00	5.05	27.86	28.74	3.07	0.712	7.00	10.00	0.97
N	8	8	8	7	7	7	7	7	7
Mean	6.750	6.524	37.645	37.074	4.325	0.5116	7.000	13.429	1.960
SD	0.8864	1.4812	10.1845	9.2881	3.0313	0.28177	0.5774	4.4293	1.3738
Minimum	5.00	4.56	24.91	25.52	1.74	0.156	6.00	10.00	0.79
Median	7.000	6.655	36.705	37.467	3.073	0.6549	7.000	10.000	1.058
Maximum	8.00	8.33	53.49	50.53	9.76	0.879	8.00	20.00	4.44
Maximum	8.00	8.33	53.49	50.53	9.76	0.879	8.00	20.00	4.44
CV%	13.1	22.7	27.1	25.1	70.1	55.1	8.2	33.0	70.1
Geometric Mean	6.694	6.372	36.443	36.075	3.544	0.4317	6.979	12.842	1.605

* Not estimated

Annexure 8

PHARMACOKINETIC DATA OF DISTACLOR CAPSULES 500mg

Annexure 8

Individual and Mean Plasma concentrations for Cefaclor after administration of one capsule of "Distaclor®" 500mg (Ranbaxy, India) under fed conditions

Time → / Subject ↓	Concentration (µg/mL)														
	0.00	**1.00**	**2.00**	**3.00**	**4.00**	**5.00**	**6.00**	**7.00**	**8.00**	**10.00**	**12.00**	**16.00**	**18.00**	**20.00**	**24.00**
1	0.00	0.26	6.44	6.20	2.46	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	2.65	6.14	6.64	2.82	1.21	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	6.91	4.64	1.55	0.52	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	1.42	4.82	5.41	3.59	2.23	1.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	1.47	4.94	7.59	3.80	1.58	0.80	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	1.21	5.45	7.64	3.55	1.97	0.76	0.38	0.24	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	1.55	6.83	4.63	1.89	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	8.52	7.83	2.67	0.97	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	1.26	5.56	7.30	3.50	1.31	0.55	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	4.95	7.02	4.09	1.81	0.66	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Mean	0.000	2.754	5.618	5.322	2.872	1.235	0.455	0.197	0.024	0.000	0.000	0.000	0.000	0.000	0.000
SD	0.0000	2.9548	1.4873	2.0839	1.7584	0.8886	0.4679	0.2212	0.0756	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Minimum	0.00	0.00	2.65	1.55	0.52	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Medium	0.00	1.445	5.503	5.778	2.981	0.984	0.391	0.119	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Maximum	0.00	8.52	7.83	7.64	6.64	2.82	1.21	0.56	0.24	0.00	0.00	0.00	0.00	0.00	0.00
CV%	-	107.3	26.5	39.2	61.2	71.9	102.8	112.1	316.2	-	-	-	-	-	-
Geometric Mean	-	-	5.409	4.826	2.331	0.930	-	-	-	-	-	-	-	-	-

Annexure 8

Individual and Mean Pharmacokinetic Parameters of Cefaclor after Administration of 1 x 500 mg Distaclor capsule under fed conditions

Subject	T_{max} (h)	C_{max} (µg/mL)	AUC_{0-t} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)	AUC_% Extrap.	λ_z (1/h)	λ_z_start (h)	λ_z_end (h)	$t_{1/2}$ (h)
1	2.00	6.44	15.65	16.15	3.35	1.178	3.00	5.00	0.59
2	4.00	6.64	19.74	20.41	3.21	0.829	4.00	7.00	0.84
3	1.00	6.91	13.73	13.93	1.37	1.038	2.00	5.00	0.67
4	3.00	5.41	18.68	19.16	2.57	0.846	5.00	7.00	0.82
5	3.00	7.59	20.37	20.92	2.65	0.703	5.00	7.00	0.99
7	3.00	7.64	21.07	21.41	1.37	0.713	3.00	8.00	0.97
8	2.00	6.83	15.20	15.78	3.83	1.026	3.00	5.00	0.68
10	1.00	8.52	20.18	20.58	1.92	0.966	3.00	5.00	0.72
11	3.00	7.30	19.59	19.87	1.40	0.852	5.00	7.00	0.81
12	2.00	7.02	18.65	18.86	1.17	1.039	4.00	6.00	0.67
N	10	10	10	10	10	10	10	10	10
Mean	2.400	7.030	18.286	18.708	2.285	0.9190	3.700	6.200	0.774
SD	0.9661	0.8256	2.5166	2.5402	0.9673	0.15496	1.0593	1.353	0.1339
Minimum	1.00	5.41	13.73	13.93	1.17	0.703	2.00	5.00	0.59
Medium	2.500	6.968	19.134	19.52	2.242	0.9088	3.500	6.500	0.766
Maximum	4.00	8.52	21.07	21.41	3.3	1.178	5.00	8.00	0.99
CV%	40.3	11.7	13.8	13.6	42.3	16.9	28.6	18.3	17.3
Geometric Mean	2.195	6.985	18.118	18.540	2.100	0.9071	3.557	6.106	0.764

Annexure 9

STABILITY DATA OF CEFACLOR OD (750mg & 1g) – 6 HOUR PROFILE

Annexure 9

Accelerated Stability Data

Product Name : **Cefaclor OD**
Strength : 750mg
Batch No. : SPB(72)157A
Description : Blue, film coated, capsule shaped, biconvex tablets

Pack	Storage Time (Months)	Parameter									
		Description	Water (%)	Assay (%)	Dissolution profile (% drug released)						
					1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
	Limits	As above	NMT 10%	90-110%	To be monitored					
	Initial	Complies	5.8	90-110	23.3	36.4	55.5	70.2	81.0	89.1
Aclar / PVC	**40°C /75%RH**									
	1 month	Complies	5.9	99.94	33.8	53.0	73.7	88.0	95.4	96.7
	2 months	Complies	5.61	101.65	44.1	63.6	85.2	96.7	98.8	-
	3 months	Complies	5.4	99.98	54.1	70.9	89.6	99.6	-	-
	25°C /60%RH									
	3months	Complies	5.0	102.07	30.9	49.3	69.1	84.7	95.1	97.7
HDPE	**40°C /75%RH**									
	1 month	Complies	5.81	100.97	32.5	50.6	70.4	84.5	93.4	96.6
	2 months	Complies	5.11	101.23	42.6	59.5	78.9	92.8	99.3	101.3
	3 months	Complies	5.04	100.51	51.3	67.1	86.5	95.1	97.0	-
	25°C /60%RH									
	3 months	Complies	4.8	101.84	29.0	47.5	73.3	90.3	97.7	99.0

Annexure 9 (contd)

Accelerated Stability Data

Product Name : **Cefaclor OD**
Strengt : 1000mg
Batch No. : SPB(72)175
Description : Blue, film coated, capsule shaped, biconvex tablets

Pack	**Storage Time (Months)**	**Parameter**									
		Description	**Water (%)**	**Assay (%)**	**Dissolution profile (% drug released)**						
					1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	
	Limits	As above	NMT 10%	90-110%	To be monitored						
	Initial	Complies			22.4	35.7	51.1	64.2	77.4	86.3	
Aclar / PVC	**40°C /75%RH**										
	1 month	Complies	5.46	98.40	32.1	56.6	81.0	89.9	90.9	-	
HDPE	**40°C /75%RH**										
	1 month	Complies	5.24	99.12	30.0	59.5	81.8	88.1	88.70	-	

Note: Study was discontinued after one month

Annexure 10

PHARMACOKINETIC DATA OF CEFACLOR OD (2x 750mg & 1x 1g) –6 HOUR PROFILE

Annexure 10

Individual and Mean Pharmacokinetic Parameters of Cefaclor after Administration of 2 x 750 mg Cefaclor OD Tablets(6 hr profile) under fed conditions

Time → / Subject↓	Concentration (µg/mL)															
	0.00	**1.00**	**2.00**	**3.00**	**4.00**	**5.00**	**6.00**	**7.00**	**8.00**	**10.00**	**12.00**	**14.00**	**16.00**	**18.00**	**20.00**	**24.00**
1	0.00	0.00	1.90	3.62	5.69	8.70	16.64	9.83	4.49	0.80	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.23	1.42	2.27	3.60	3.64	9.06	6.39	2.41	0.27	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.33	1.75	1.91	8.44	13.23	17.25	16.40	7.43	1.37	0.33	0.00	0.00	0.00	0.00	0.00
4	0.00	0.57	2.14	2.28	6.92	4.07	8.22	5.08	2.27	0.47	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	1.72	4.68	5.78	7.49	9.25	6.43	5.24	2.00	0.37	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.26	1.23	1.25	5.42	12.00	18.98	14.61	6.87	1.15	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.47	0.80	1.38	4.29	10.79	9.74	6.32	3.55	1.05	0.26	0.00	0.00	0.00	0.00	0.00
N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Mean	0.000	0.478	1.960	2.730	6.010	8.782	12.124	9.031	4.233	0.834	0.114	0.000	0.000	0.000	0.000	0.000
SD	0.0000	0.5311	1.1769	1.4938	1.6130	3.4451	4.7582	4.3232	2.0769	0.4175	0.1589	0.0000	0.0000	0.0000	0.0000	0.0000
Minimum	0.00	0.00	0.80	1.25	3.60	3.64	6.43	5.08	2.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00
Median	0.000	0.293	1.756	2.271	5.970	8.975	10.209	7.388	4.019	0.924	0.000	0.000	0.000	0.000	0.000	0.000
Maximum	0.00	1.72	4.68	5.78	8.44	13.23	18.98	16.40	7.43	1.37	0.33	0.00	0.00	0.00	0.00	0.00
CV%	-	111.1	60.0	54.7	26.8	39.2	39.2	47.9	49.1	50.1	139.2	-	-	-	-	-
Geometric Mean	-	-	1.735	2.424	5.810	8.044	11.328	8.238	3.796	0.723	-	-	-	-	-	-

Annexure 10

Individual and Mean Pharmacokinetic Parameters of Cefaclor after Administration of 2 x 750 mg Cefaclor OD Tablets(6 hr profile) under fed conditions

Subject	T_{max} (h)	C_{max} (µg/mL)	AUC_{0-t} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)	AUC_% Extrap.	λ_z (1/h)	λ_z_start (h)	λ_z_end (h)	$t_{1/2}$ (h)
1	6.00	16.64	53.89	54.84	1.7	0.841	7.00	10.00	0.82
2	6.00	9.06	30.49	30.74	0.8	1.058	7.00	10.00	0.66
3	6.00	17.25	73.51	73.94	0.6	0.787	7.00	12.00	0.88
4	6.00	8.22	33.16	33.76	1.8	0.790	7.00	10.00	0.88
5	5.00	9.25	43.96	44.39	1.0	0.876	7.00	10.00	0.79
7	6.00	18.98	65.19	66.53	2.0	0.855	7.00	10.00	0.81
8	5.00	10.79	41.48	41.89	1.0	0.637	7.00	12.00	1.09
N	8	8	8	8	8	8	8	8	8
Mean	5.750	12.608	48.865	49.473	1.23	0.8152	-	-	0.869
SD	0.4629	4.2845	14.9401	15.1149	0.529	0.12911	-	-	0.1356
Minimum	5.00	8.22	30.49	30.74	0.57	0.637	-	-	0.66
Medium	6.000	10.733	46.597	47.05	0.965	0.8158	-	-	0.851
Maximum	6.00	18.98	73.51	73.94	2.01	1.058	-	-	1.09
CV%	8.1	34.0	30.6	30.6	43.2	15.8	-	-	15.6
Geometric Mean	5.733	12.009	46.908	47.490	1.128	0.8065	-	-	0.859

Annexure 10

Individual and Mean Pharmacokinetic Parameters of Cefaclor after Administration of 1 x 1.0 g Cefaclor OD Tablets (6 hr profile) under fed conditions

Time → / Subject↓	Concentration (µg/mL)															
	0.00	**1.00**	**2.00**	**3.00**	**4.00**	**5.00**	**6.00**	**7.00**	**8.00**	**10.00**	**12.00**	**14.00**	**16.00**	**18.00**	**20.00**	**24.00**
9	0.00	0.25	1.31	4.33	6.08	13.38	10.73	5.21	1.73	0.30	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	1.01	8.02	6.40	5.62	6.98	3.71	1.56	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.46	1.26	2.41	2.15	3.02	5.17	5.69	3.47	1.92	2.23	1.01	0.34	0.27	0.00	0.00
12	0.00	0.23	1.40	6.05	5.17	9.21	5.10	4.61	1.97	0.29	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	1.04	2.94	9.20	8.10	6.14	5.20	4.47	1.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.65	4.05	12.27	8.53	5.60	4.04	1.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.60	1.59	2.42	7.61	5.84	4.90	3.39	0.96	0.22	0.00	0.00	0.00	0.00	0.00
16	0.00	0.96	1.33	1.82	7.58	13.70	5.07	1.96	0.85	0.22	0.00	0.00	0.00	0.00	0.00	0.00
N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Mean	0.000	0.494	2.188	4.481	6.174	8.570	5.802	4.054	1.824	0.462	0.306	0.126	0.043	0.034	0.000	0.000
SD	0.0000	0.4475	2.4632	2.6276	3.2617	3.5870	2.0873	1.5021	1.1050	0.6683	0.7819	0.3571	0.1206	0.0965	0.0000	0.0000
Minimum	0.00	0.00	0.00	0.60	1.59	2.15	3.02	3.71	1.56	0.44	0.00	0.00	0.00	0.00	0.00	0.00
Median	0.000	0.355	1.318	4.191	5.849	8.067	5.183	4.538	1.657	0.255	0.000	0.000	0.000	0.000	0.000	0.000
Maximum	0.00	1.04	8.02	9.20	12.27	13.70	10.73	5.69	3.47	1.92	2.23	1.01	0.34	0.27	0.00	0.00
CV%	-	90.5	112.6	58.6	52.8	41.9	36.0	37.0	60.6	144.8	255.4	282.8	282.8	282.8	-	-
Geometric Mean	-	-	1.518	3.801	5.371	7.833	5.553	3.723	1.514	-	-	-	-	-	-	-

Annexure 10

Individual and Mean Pharmacokinetic Parameters of Cefaclor after Administration Cefaclor OD, 1.0g tablets (6hr profile) under fed conditions

Subject	T_{max} (h)	C_{max} (µg/mL)	AUC_{0-t} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)	AUC_% Extrap.	λ_z (1/h)	λ_z_start (h)	λ_z_end (h)	t1/2 (h)
9	5.00	13.38	44.18	44.51	0.7	0.909	6.00	10.00	0.76
10	2.00	8.02	33.51	33.92	1.2	1.067	6.00	8.00	0.65
11	7.00	5.69	36.64	37.65	2.7	0.271	7.00	18.00	2.56
12	5.00	9.21	35.01	35.33	0.9	0.925	7.00	10.00	0.75
13	3.00	9.20	37.87	43.04	12.0	0.307	3.00	8.00	2.26
14	4.00	12.27	35.72	37.87	5.7	0.545	4.00	8.00	1.27
15	5.00	7.61	30.20	30.51	1.0	0.687	8.00	12.00	1.01
16	5.00	13.70	33.91	34.21	0.9	0.725	7.00	10.00	0.96
N	8	8	8	8	8	8	8	8	8
Mean	4.500	9.884	35.880	37.128	3.14	0.6794	-	-	1.277
SD	1.5119	2.9211	4.0725	4.7227	3.948	0.28997	-	-	0.7282
Minimum	2.00	5.69	30.20	30.51	0.74	0.271	-	-	0.65
Medium	5.000	9.201	35.366	36.49	1.124	0.7060	-	-	0.982
Maximum	7.00	13.70	44.18	44.51	12.00	1.067	-	-	2.56
CV%	33.6	29.6	11.4	12.7	125.8	42.7	-	-	57.0
Geometric Mean	4.243	9.496	35.686	36.871	1.844	0.6142	-	-	1.129

Annexure 11

STABILITY DATA OF CEFACLOR OD (750mg) – 10 HOUR PROFILE

Annexure-11
Accelerated Stability Data

Product Name : **Cefaclor OD**
Strength : 750mg
Batch No. : SPB(99)63
Pack : Aluminium strips
Description : Blue, film coated, capsule shaped, biconvex tablets

Storage Time (Months)	Parameter											
	Description	Water (%)	Assay (%)	Dissolution profile (% drug released)								
				1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	9 hrs	10 hrs
Limits	As above	NMT 10%	90-110%	To be monitored									
Initial	Complies	5.06	103.24	17.1	21.4	30.4	39.0	47.4	56.0	64.6	72.8	80.3	87.5
40°C /75%RH													
1 month	Complies	5.11	99.63	20.1	27.6	36.5	45.2	54.5	63.8	72.8	81.7	88.7	95.2
3 months	Complies	5.14	98.77	19.1	26.1	36.2	45.9	55.5	64.3	73.0	82.1	89.0	95.3
25°C /60%RH													
3months	Complies	4.98	100.95	16.5	24.0	34.7	43.2	51.3	60.1	68.8	75.6	84.6	91.8

Annexure 12

PHARMACOKINETIC DATA OF CEFACLOR OD (2x 750mg) –10 HOUR PROFILE

Annexure 12

Individual and Mean Plasma Concentrations for Cefaclor after administration of two tablets of Cefaclor OD 750 mg (10 hr profile) under fed conditions

Time → / Subject ↓	Concentration (µg/mL)														
	0.00	**1.00**	**2.00**	**3.00**	**4.00**	**5.00**	**6.00**	**7.00**	**8.00**	**10.00**	**12.00**	**16.00**	**18.00**	**20.00**	**24.00**
1	0.00	1.00	2.34	2.20	3.48	5.52	5.46	3.22	1.36	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	2.84	3.42	3.33	2.61	3.12	1.97	9.25	5.63	4.78	5.52	1.08	0.00	0.00	0.00
3	0.00	1.01	2.00	1.95	2.64	3.04	3.32	4.63	3.35	1.77	2.15	0.34	0.00	0.00	0.00
4	0.00	1.11	2.07	2.16	2.15	5.14	7.10	3.49	1.93	2.48	2.23	1.66	3.36	1.21	0.00
5	0.00	1.23	3.61	3.03	5.72	4.54	8.66	6.48	4.02	1.45	1.17	0.00	0.29	0.21	0.00
7	0.00	0.87	2.99	1.14	1.29	8.75	7.65	9.16	2.90	0.38	0.00	0.00	0.00	0.00	0.00
8	0.00	0.50	1.60	1.68	2.88	5.47	5.45	6.26	5.11	2.27	2.48	0.63	0.00	0.00	0.00
10	0.00	2.29	2.20	2.24	1.02	8.03	6.60	6.25	3.06	0.57	0.00	0.00	0.00	0.00	0.00
11	0.00	1.72	2.47	1.94	2.87	4.20	6.58	6.15	3.17	2.09	20.1	4.05	1.40	1.95	0.96
12	0.00	1.84	2.02	1.85	3.12	4.20	7.97	7.74	6.27	1.75	1.21	0.92	0.33	0.00	0.00
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Mean	0.000	1.442	2.472	2.151	2.778	5.202	6.075	6.263	3.681	1.754	1.675	0.867	0.538	0.338	0.096
SD	0.0000	0.7209	0.6572	0.6321	1.2915	1.8917	2.0967	2.0853	1.5800	1.3553	1.6647	1.2532	1.0825	0.6821	0.3020
Minimum	0.00	0.50	1.60	1.14	1.02	3.04	1.97	3.22	1.36	0.00	0.00	0.00	0.00	0.00	0.00
Medium	0.000	1.170	2.271	2.053	2.754	4.842	6.590	6.253	3.260	1.760	1.606	0.483	0.000	0.000	0.000
Maximum	0.00	2.84	3.61	3.33	5.72	8.75	8.66	9.25	6.27	4.78	5.52	4.05	3.36	1.95	0.96
CV%	-	50.0	26.6	29.4	46.5	36.4	34.5	33.3	42.9	77.3	99.4	144.6	201.3	202.0	316.2
Geometric Mean	-	1.286	2.398	2.068	2.513	4.919	5.623	5.924	3.354	-	-	-	-	-	-

Annexure 12

Individual and Mean Pharmacokinetic Parameters of Cefaclor after Administration of 2 x 750 mg OD tablets (10hr profile) under fed conditions

Subject	T_{max} (h)	C_{max} (µg/mL)	AUC_{0-t} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)	AUC_% Extrap.	λ_z (1/h)	λ_z_start (h)	λ_z_end (h)	$t_{1/2}$ (h)
1	5.00	5.52	23.90	25.86	7.97	0.695	6.00	8.00	1.00
2	7.00	9.25	63.25	68.47	9.67	0.207	7.00	16.00	3.34
3	7.00	4.63	34.26	35.51	4.39	0.270	7.00	16.00	2.57
4	6.00	7.10	50.68	69.59	32.21	0.064	6.00	20.00	10.83
5	6.00	8.66	46.50	47.48	1.94	0.218	12.00	20.00	3.18
7	7.00	9.16	36.57	36.93	0.94	1.059	7.00	10.00	0.65
8	7.00	6.26	44.74	47.30	5.86	0.245	7.00	16.00	2.82
10	5.00	8.03	33.79	34.50	2.08	0.806	7.00	10.00	0.86
11	6.00	6.58	63.62	75.21	17.29	0.082	6.00	24.00	8.41
12	6.00	7.97	48.33	49.59	2.76	0.260	6.00	18.00	2.67
N	10	10	10	10	10	10	10	10	10
Mean	6.200	7.315	44.564	49.042	8.513	0.3906	7.100	15.800	3.635
SD	0.7888	1.5645	12.8262	16.8947	9.6565	0.33825	1.7920	5.1164	3.3531
Minimum	5.00	4.63	23.90	25.86	0.94	0.064	6.00	8.00	0.65
Medium	6.000	7.534	45.619	47.39	5.125	0.2525	7.000	16.000	2.747
Maximum	7.00	9.25	63.62	75.21	32.21	1.059	12.00	24.00	10.83
CV%	12.7	21.4	28.8	34.4	113.4	86.6	25.2	32.4	92.2
Geometric Mean	6.153	7.153	42.825	46.442	5.088	0.2745	6.946	14.973	2.526

Annexure 13

PHARMACOKINETIC DATA OF CEFACLOR OD (2x 500mg)

–10 HOUR PROFILE WITH PROBENECID

Annexure 13

Individual and Mean Plasma Concentrations for Cefaclor after administration of Two Tablets of Cefaclor OD 500 mg(10hr profile) with Two Tablets of Probenecid 500 mg under fed conditions

Time → / Subject ↓	Concentration (µg/mL)														
	0.00	**1.00**	**2.00**	**3.00**	**4.00**	**5.00**	**6.00**	**7.00**	**8.00**	**10.00**	**12.00**	**16.00**	**18.00**	**20.00**	**24.00**
1	0.00	0.00	3.01	3.35	4.41	7.87	9.49	8.13	3.29	0.57	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	2.29	3.24	1.97	3.19	4.07	7.38	5.82	4.30	2.65	1.53	1.35	3.72	1.87
3	0.00	1.67	2.44	3.15	3.49	3.82	3.64	7.96	4.63	2.16	0.51	0.00	0.00	0.00	0.00
4	0.00	0.62	1.51	2.25	3.06	3.79	9.03	6.22	3.66	2.65	2.26	0.80	0.73	1.41	1.54
5	0.00	1.31	2.92	3.24	5.89	9.38	9.06	10.30	6.84	3.81	2.00	2.84	1.44	2.78	0.45
7	0.00	2.15	2.69	2.02	3.28	5.84	12.32	12.72	7.63	2.34	0.51	0.00	0.00	0.00	0.00
8	0.00	0.70	1.24	1.97	3.50	6.19	6.81	8.34	7.47	2.54	1.04	0.61	0.60	1.63	0.00
10	0.00	1.27	3.17	3.91	5.04	7.99	11.24	10.39	5.12	1.11	0.21	0.00	0.00	0.00	0.00
11	0.00	0.56	2.66	3.86	4.03	5.76	4.62	6.72	4.45	3.26	1.89	2.11	2.38	4.23	0.36
12	0.00	1.16	3.96	3.48	3.70	5.43	8.28	8.06	5.57	3.64	2.02	1.04	0.31	0.00	0.00
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Mean	0.000	0.943	2.588	3.047	3.837	5.927	7.855	8.622	5.447	2.636	1.309	0.893	0.682	1.376	0.421
SD	0.0000	0.6956	0.7903	0.7158	1.0883	2.0229	2.9954	1.9662	1.5116	1.1768	0.9611	0.9990	0.8119	1.6713	0.7003
Minimum	0.00	0.00	1.24	1.97	1.97	3.19	3.64	6.22	3.29	0.57	0.00	0.00	0.00	0.00	0.00
Medium	0.000	0.927	2.675	3.238	3.598	5.800	8.654	8.094	5.343	2.598	1.467	0.705	0.454	0.704	0.000
Maximum	0.00	2.15	3.96	3.91	5.89	9.38	12.32	12.72	7.63	4.30	2.65	2.84	2.38	4.23	1.87
CV%	-	73.8	30.5	23.5	28.4	34.1	38.1	22.8	27.8	44.6	73.4	111.9	119.0	121.4	166.3
Geometric Mean	-	-	2.463	2.962	3.693	5.609	7.273	8.436	5.254	2.299	-	-	-	-	-

Annexure 13

Individual and Mean Pharmacokinetic Parameters of Cefaclor after Administration of 2 x 500 mg OD tablets (10hr profile) under fed conditions

Subject	T_{max} (h)	C_{max} (µg/mL)	AUC_{0-t} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)	AUC_% Extrap.	λ_z (1/h)	λ_z_start (h)	λ_z_end (h)	$t_{1/2}$ (h)
1	6.00	9.49	41.74	42.38	1.50	0.888	7.00	10.00	0.78
2	7.00	7.38	69.58	94.47	21.99	0.075	7.00	24.00	9.25
3	7.00	7.96	37.93	38.88	2.78	0.534	7.00	12.00	1.30
4	6.00	9.03	55.22	69.43	10.74	0.108	6.00	24.00	6.39
5	7.00	10.30	86.62	89.86	5.61	0.138	7.00	24.00	5.02
7	7.00	12.72	57.65	58.44	1.48	0.643	7.00	12.00	1.08
8	7.00	8.34	52.82	62.36	6.07	0.171	7.00	20.00	4.06
10	6.00	11.24	53.11	53.37	0.50	0.798	8.00	12.00	0.87
11	7.00	6.72	71.58	74.90	10.38	0.107	7.00	24.00	6.49
12	6.00	8.28	59.19	60.39	2.73	0.259	6.00	18.00	2.68
N	10	10	10	10	10	10	10	10	10
Mean	6.600	9.146	58.543	64.449	6.379	0.3721	6.900	18.000	3.791
SD	0.5164	1.8398	14.3821	18.2633	6.5661	0.31332	0.5676	5.9628	2.9409
Minimum	6.00	6.72	37.93	38.88	0.50	0.075	6.00	10.00	0.78
Medium	7.000	8.684	56.432	61.37	4.196	0.2148	7.00	19.000	3.370
Maximum	7.00	12.72	86.62	94.47	21.99	0.888	8.00	24.00	9.25
CV%	7.8	20.1	24.6	28.3	102.9	84.2	8.2	33.1	77.6
Geometric Mean	6.581	8.988	56.983	62.111	3.847	0.2577	6.879	17.040	2.690

Annexure 14

PROPOSED SPECIFICATIONS AND STANDARD TESTING PROCEDURE FOR BID PRODUCT

PROPOSED SPECIFICATIONS FOR CEFACLOR BID TABLETS

S.No.	Test	Specification		
1.	Description	Blue coloured, capsule shaped, biconvex film coated tablets.		
2.	Identification	Test A and B to comply		
3.	Uniformity of weight (%)	± 5% of average weight		
4.	Water by KF (%w/w) (Use 0.5 g sample)	NMT 7.0		
5.	Dissolution (% of labeled amount of Cefaclor ($C_{15}H_{14}Cl\ N_3O_4S$) dissolved) (900mL media , Apparatus USP-1, 100 RPM) Media - 0.1N HCl (0 - 1 Hr) , Phosphate Buffer pH 6.8 (1-4 Hr)	In 60 minutes 20 – 50 In 120 minutes 45 -75 In 180 minutes 70 – 95 In 240 minutes NLT 85		
6.	Assay Each tablet contains Cefaclor I.P. equivalent to anhydrous Cefaclor (mg)	**Limits** 450.0 – 550.0 (90.0-110.0% of actual input)	**Claim** 500	**Actual Input** 500

Storage Condition: Store below 25°C in a dry place. Protect from light.

Shelf Life : 24 months

PROPOSED STANDARD TESTING PROCEDURE FOR CEFACLOR BID TABLETS

IDENTIFICATION

A) Test preparation: Transfer an accurately weighed portion of the powdered tablets equivalent to about 30 mg of Cefaclor to a 100 mL volumetric flask. Add about 70 mL of water, sonicate for 5 minutes and make up the volume with water and mix. Filter through Whatman filter paper No. 41. Discard first few mL of filtrate. Further dilute 1 mL of filtrate to 100 mL with water and mix.

Procedure : Measure the light absorption of test preparation in the range 190 nm to 310 nm against water as blank.

Inference: Light absorption exhibits a maximum only at 264 nm.

B) In the assay, the chromatogram obtained with test solution, shows a peak with the same retention time as the principal peak in the chromatogram obtained with standard solution.

DISSOLUTION

Dissolution parameters :

Apparatus : USP Type 1

RPM : 100

Medium : 0.1 N Hydrochloric acid; 900 mL upto end of 1st hour, pH 6.8 phosphate buffer; 900mL from end of 1st hour upto end of 4th hour

Time : 240 minutes

Volume withdrawn : 10 mL

Phosphate buffer : Dissolve 6.8 g of potassium dihydrogen phosphate and 0.89 g of sodium hydroxide in purified water and make upto 1000 mL with purified water. If necessary, adjust to pH 6.8 with respective medium.

Diluent : Dissolve 4.5 gm of potassium dihydrogen orthophosphate in purified water and make upto 1000 mL with purified water.

Standard preparation : Weigh accurately about 55 mg of Cefaclor WS in a 100 mL of volumetric flask, dissolve in 75 mL of diluent , sonicate if necessary and dilute to 100 mL with diluent and mix. Filter through Whatman filter paper No. 41. Discard first few mL of filtrate. Further dilute 10 mL of filtrate to 250 mL with diluent and mix.

Discard first few mL of filtrate. Further dilute 10 mL of filtrate to 250 mL with diluent and mix.

Test solution: At each hour or at the specified time, withdraw 10 mL of aliquot. Filter through Whatman filter paper No. 41. Discard first few mL of filtrate. Further dilute 5 mL of filtrate to 50 mL with diluent and mix.

Procedure : Measure the absorbance of standard and test preparation at 264 nm against diluent as blank.

Calculation : Calculate the % Cefaclor ($C_{15}H_{14}Cl\,N_3O_4S$) dissolved at each hour as follows:

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{LC} \times \frac{P}{100} \times 100$$

Where,

AT = Absorbance of test preparation

AS = Absorbance of standard preparation

WS = Weight of standard in mg.

DS = Dilution of standard preparation

DT = Dilution of test preparation

P = Potency of standard (as is basis)

LC = Label Claim (mg/tablets)

Calculate cumulative % of Cefaclor dissolved.

ASSAY

Instrumental Conditions :

Column	: Hypersil ODS, 250 x 4.6 mm, 5µ
Flow Rate	: 1.5mL/min.
Wavelength	: 265 nm
Injection Volume	: 20µl
Run time	: 15 minutes
Column temperature	: Ambient

Mobile phase preparation : Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of purified water and 10 mL of triethylamine. Adjust with phosphoric acid to pH 2.5 ± 0.05; add 220 mL of methanol and mix. Filter and degas.

Resolution solution : Prepare a solution in mobile phase containing about 0.3 mg of Cefaclor WS and 0.3 mg of delta-3-Cefaclor per mL.

Standard preparation : Weigh accurately about 30 mg of Cefaclor WS to a 100 mL volumetric flask. Add about 70 mL of mobile phase and sonicate if necessary to dissolve and dilute to volume with mobile phase and mix (avoid heating solution). [Note : use this solution within 8 hours if stored at room temperature or within 20 hours if stored under refrigeration].

Test preparation :

For blend: Transfer an accurately weighed portion of the powder equivalent to about 75 mg of Cefaclor to a 250 mL volumetric flask. Add about 200 mL of mobile phase, sonicate for 5 minutes in cold water with constant swirling and make up the volume with mobile phase and mix.

For Finished product: Weigh and finely powder NLT 20 tablets and proceed further from "transfer an accurately weighed ....." as mentioned under blend.

Procedure : Filter the resolution solution, standard and test preparation through 0.45 μ membrane filter. Inject resolution solution in single, standard in replicate and test in duplicate. Calculate system suitability parameters from resolution chromatogram and % RSD from standard chromatograms as mentioned below:

Relative retention time : 0.8 for Cefaclor and 1.0 for Delta-3-Cefaclor

Resolution (R) : NLT 2.5 between Cefaclor and Delta-3-Cefaclor

Tailing factor :NMT 1.5 for Cefaclor

% RSD : NMT 2.0 for replicate injections.

Calculation : Calculate the content of Cefaclor as mg/tablet as follows:

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt.}$$

Where,

AT = Average area of test preparation

AS = Average area of standard preparation

WS = Weight of standard in mg

WT = Weight of test in mg

DS = Dilution of standard preparation

DT = Dilution of test preparation

P = Potency of standard (as is basis)

Avg. Wt. = Average weight of tablet (mg/tablet)

Annexure 15

PROPOSED SPECIFICATIONS AND STANDARD TESTING PROCEDURE FOR OD PRODUCT

PROPOSED SPECIFICATIONS FOR CEFACLOR OD TABLETS

S.No.	Test	Specification	
1.	Description	Blue coloured , capsule shaped , biconvex , film coated tablets.	
2.	Identification	The retention time of the major peak in the chromatogram obtained in the assay preparation corresponds to that in the chromatogram of standard preparation.	
3.	Water by KF (%w/w)	NMT 6	
4.	Uniformity of dosage units (By weight variation)	To meet the requirement	
5.	Dissolution (% of labeled amount of Cefaclor ($C_{15}H_{14}Cl\ N_3O_4S$) dissolved) (900mL media , Apparatus USP-1, 100 RPM) Media - 0.1N HCl (0 - 2 Hr) , Phosphate Buffer pH 6.8 (2-10 Hr)	Time (Hours) 1 2 4 8 10	Limit (%) 10 - 30 20 - 40 35 - 55 70 - 90 NLT 85
6.	Assay Each film coated tablet contains Cefaclor	**Limit** 675.0 – 825.0 mg 90 - 110 % of label claim	**Label claim** 750 mg

Storage Condition: Store below 25°C in a dry place. Protect from light.

Shelf Life : 24 months

PROPOSED STANDARD TESTING PROCEDURE FOR CEFACLOR OD TABLETS

IDENTIFICATION

The retention time of the cefaclor peak in the chromatogram of test preparation corresponds to that of standard preparation as obtained in assay.

DISSOLUTION

Dissolution parameters :

Apparatus	: USP Type 1 (Basket)
RPM	: 100
Medium	: 0.1 N Hydrochloric acid; 900 mL upto end of 2nd hour, pH 6.8 phosphate buffer; 900mL from end of 2nd hour onwards
Time	: 10 hours
Volume withdrawn	: 20 mL

Phosphate buffer : Dissolve 6.8 g of potassium dihydrogen phosphate in purified water and make upto 1000 mL with purified water. Adjust pH to 6.8 with 1N sodium hydroxide.

Diluent : Dissolve 4.5 gm of potassium dihydrogen orthophosphate in purified water and make upto 1000 mL with purified water

Standard preparation : Weigh accurately about 55 mg of Cefaclor WS in a 100 mL volumetric flask. Dissolve in 75 mL of diluent , sonicate if necessary and dilute to 100 mL with diluent and mix. Filter through Whatman filter paper No. 41. Discard first few mL of filtrate. Further dilute 10 mL of filtrate to 250 mL with diluent and mix.

Test solution: At each hour or at the specified time, withdraw 10 mL of aliquot. Filter through Whatman filter paper No. 41. Discard first few mL of filtrate. Further dilute 5 mL of filtrate to 50 mL with diluent and mix.

Procedure : Measure the absorbance of standard and test preparation at 264 nm against diluent as blank.

Calculation : Calculate the % Cefaclor ($C_{15}H_{14}Cl\ N_3O_4S$) dissolved at each hour as follows:

$$\frac{AT}{AS} \times \frac{DS}{DT} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = Absorbance of test preparation

AS = Absorbance of standard preparation

DS = Dilution of standard preparation

DT = Dilution of test preparation

P = Potency of standard (as is basis)

LC = Label Claim (mg/tablets)

Calculate cumulative percentage of Cefaclor dissolved .

ASSAY

Instrumental Conditions :

Column	: Hypersil ODS, 250 x 4.6 mm, 5µ
Flow Rate	: 1.5mL/min.
Wavelength	: 265 nm
Injection Volume	: 20µl
Run time	: 15 minutes
Column temperature	: Ambient

Mobile phase preparation : Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of purified water and 10 mL of triethylamine. Adjust with phosphoric acid to pH 2.5 ± 0.05; add 220 mL of methanol and mix. Filter and degas.

Resolution solution : Accurately weigh and transfer about 30mg of Cefaclor WS and 30mg of Delta-3-Cefaclor in a 100 mL volumetric flask. Add 70mL of mobile phase and sonicate to dissolve. Dilute up to mark with mobile phase and mix.

Standard preparation : Weigh accurately about 30 mg of Cefaclor WS to a 100 mL volumetric flask. Add about 70 mL of mobile phase and sonicate to dissolve and dilute to volume with mobile phase and mix . [Note : use this solution within 8 hours if stored at room temperature or within 20 hours if stored under refrigeration].

Test preparation : Weigh and crush NLT 20 tablets to fine powder. Accurately weigh and transfer about 75mg equivalent of cefaclor to a 250mL volumetric flask. Add 200mL mobile phase and sonicate for 10 minutes in cold water. Make up the volume with mobile phase and mix.

Procedure : Filter the resolution solution, standard and test preparation through 0.45 μ membrane filter. Inject resolution solution in single, standard in replicate and test in duplicate. The test should meet the following system suitability requirements in chromatograms of resolution solution and requirement of RSD in the chromatogram of standard preparation:

Relative retention time	: 0.8 for Cefaclor and 1.0 for Delta-3-Cefaclor
Resolution (R)	: NLT 2.5 between Cefaclor and Delta-3-Cefaclor
Tailing factor	: NMT 1.5 for Cefaclor
% RSD	: NMT 2.0 for replicate injections.

Calculation : Calculate the content of Cefaclor as mg/tablet as follows:

$$\frac{AT}{AS} \times \frac{DS}{DT} \times \frac{P}{100} \times W$$

Where,

AT = Average area of test preparation

AS = Average area of standard preparation

DS = Dilution of standard preparation

DT = Dilution of test preparation

P = Potency of standard (as is basis)

W = Average weight of tablet (mg/tablet)